THE UNIVERSITY OF ALBERTA

ALTERATIONS IN THE COMPOSITION OF ORGANIC MATTER AS RELATED TO PODZOLIC DEGRADATION OF CHERNOZEMIC SOILS

A DISSERTATION

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

DEPARTMENT OF SOIL SCIENCE

by

C. D. SAWYER, B.Sc.
EDMONTON, ALBERTA

APRIL, 1962

ABSTRACT

The soils of Northern Alberta are dominantly Grey Wooded while those in the southern part of the Province are largely Chernozemic. The transitional area between these two regions is characterized by Dark Grey Wooded and Dark Grey Chernozemic soils. These soils are common to the parkland areas and are postulated to have been formerly characterized by a grassland vegetation, and were only recently invaded by deciduous forests. Concomitant with this forest invasion has been the degradation of the Chernozemic soils to Dark Grey Chernozemic and Dark Grey Wooded soils.

The purpose of this investigation was to study the properties of the soil organic constituents associated with the degradation of chernozemic A horizons, postulating that the results would aid in clarifying the degradation processes active in the genesis of Grey Wooded soils.

For this purpose, duplicate profiles of a typical Dark Grey Wooded soil and a Dark Grey Wooded soil showing morphological modifications indicative of active solonetzic processes were chosen. These profiles were sampled in the L horizon, the F-H horizon and every four centimeters through the mineral A horizon. Humic Acids were extracted from these soil samples, purified, and functional group analyses conducted. Dilute sulfuric acid extractions were analyzed for polyuronide and polysaccharide content.

Polysaccharide and polyuronide content declined rapidly with depth through the mineral A horizon; however, the percentage of total carbon present as polysaccharide and

polyuronide carbon was relatively constant. Methoxyl content of the purified humic acids decreased with depth through the mineral A horizon while the total acidity increased with depth. This increase in total acidity was evenly distributed between phenolic and carboxyl acidity. The purified humic acids showed an increase in ash content with depth through the mineral A horizon. This ash proved to be amorphous and consisted mainly of sesquioxides and silica.

It appears from the results of this study that neither humic acids, polysaccharides, nor polyuronides are responsible for the mobilization of clay minerals in the degradation processes active in Dark Grey Wooded Ah horizons. The role of humic acids may be primarily a stabilizing influence while the influence of polyuronides and polysaccharides is still conjectural.

ACKNOWLEDGEMENTS

Sincere appreciation is extended to Dr. S. Pawluk, under whose guidance this study was undertaken and conducted. Thanks are also extended to Dr. J. C. Wood, Research Council of Alberta, for his helpful advice in carrying out some of the laboratory analyses and for serving on the committee. Further thanks are extended to Dr. J. F. Dormaar for inspiration, constructive criticism and translation of some of the literature.

Appreciation is also extended to the following:

Dr. J. A. Toogood, Professor and Head of the Department of Soil Science, and his staff for assistance and encouragement;

Dr. R. J. St. Arnaud, Assistant Professor of Soil Science, University of Saskatchewan, for determining a differential thermal analysis;

Dr. W. E. Harris, Associate Professor, Department of Chemistry, for serving on the committee;

Mr. J. A. Campbell, Chief Grazing Appraiser, Department of Lands and Forests, Government of the Province of Alberta, for assistance in the identification of some of the vegetative species;

Miss Nola Pierce for typing the manuscript;

The Research Council of Alberta for making their laboratories available for some of the analyses and for carrying out infrared analyses;

Government of the Province of Alberta, Department of Lands and Forests for financial assistance and for Leave of Absence to undertake this study;

National Research Council of Canada for financial assistance.

The author would like to extend deep appreciation to his wife, Hazel, for her great patience and many sacrifices while this study was being undertaken.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

I. INTRODUCTION

Examination of a soil zone map of Alberta reveals that the soils of southern and central Alberta are predominantly chernozemic whereas the soils dominant in northern areas are Grey Wooded. Numerous intergrades between Chernozemic and Grey Wooded soils, including the Dark Grey Wooded and the Dark Grey Chernozemic may be found in the transition area between these two major regions.

It has been postulated that shortly after the last glaciation in Alberta, a xerothermic period occurred which was characterized by more arid climatic conditions than exist at present (58). It has been further postulated that during this relatively dry period, chernozemic soils with a grassland vegetation cover were established in regions lying adjacent to the northern limits of the Black Soil Zone as presently outlined. Shortly after this xerothermic period, conditions became more humid and a forest invasion from the north occurred. Prior to settlement, this invasion of the parkland area of Alberta was partially suppressed by factors such as burning, grazing, rabbit infestations, etc. Settlement of the West has meant that some of these factors have been partially eliminated and forest invasion has therefore been enhanced.

Concomitant with the tree invasion, has been the degradation of the soil. It appears that this degradation is initiated at the bottom of the A horizon and slowly progresses upward as leaching processes proceed. The Chernozemic

A horizon becomes degraded into a siliceous, eluviated, platy Ae horizon and the illuviated material gives rise to the formation of an underlying Bt horizon. The Dark Grey Chernozemic soil shows the initial stages of this type of degradation while the Dark Grey Wooded soil shows the more advanced stages. Newton et al (61) suggested that the Ah horizons of these soils were formed under grassland vegetation and the leached layers were formed after tree invasion. If the leached layers have developed since tree invasion, then it follows that some effect of the tree cover has initiated their formation. The formation of the leached layers is a degradation process. The feature of the forest cover usually attributed to causing the degradation process is the leachates or effluents of the decomposing leaf litter.

A group of soils having similar characteristics except that the B horizons shows a columnar structure indicative of solonetzic features, appear to be associated with the Dark Grey Chernozemic and Dark Grey Wooded soils of Alberta. On account of the nature of the B horizon, these soils appear to have been produced by the action of rather weak solonetzic processes which in the early stages of development may have had at least a weakly alkaline reaction. The solonetzic processes may, in fact, be partially responsible for the formation of the eluviated horizon, however, the degradation process instrumental in the formation of Dark Grey Wooded soils was also possibly effective. The soil therefore appears to be an intergrade between the Dark Grey Wooded and the Eluviated Chernozem.

The mechanism of soil degradation under forest cover are not fully understood. It is, however, known that the effluents of the decomposing tree litter have a marked effect on the mineral constituents of the soil solum. Under these leaching conditions, the colloidal complexes appear to become unstable and clay is mobilized. The mobilization of the clay colloids may, at least in part, lead to degradation as may be observed in many of our soils found under tree cover in the forest tension zone of Alberta.

The purpose of this research project is to study and compare the composition of organic matter found in various soil horizons of two Dark Grey Wooded soils common to northern parkland regions. The first of the Dark Grey Wooded soils is typical while the second shows slight morphological modifications attributed to minor solonetzic processes. The second soil may be regarded as the previously mentioned Dark Grey Wooded-Eluviated Chernozem intergrade. It is postulated that chemical analysis of the organic matter from the upper soil horizons will show characteristic changes indicative of the alterations of the organic constituents as degradation proceeds. It is further postulated that the organic matter characterizations will further elucidate the mechanism of the degradation processes active in soils common to the forest-parkland transition zone of the Province.

II. LITERATURE REVIEW

A. Introduction to Literature Review

It was not until relatively recent times that scientists fully appreciated the concept of the soil solum as we understand it today. For instance, early Danish geologists held the view that the ashy grey to white horizon in some of the forest soils in Scandinavia did not develop in situ but was deposited by wind or water. They considered the leached horizon as an independent geological stratum. The explanation of the occurrence of this bleached layer, now referred to as the Ae horizon, was not fully elucidated until the Russian Dokuchaev School of Pedology revealed the relationships of the genetics of the soil solum (45).

Dokuchaev initially proposed that the soil is a result of the combined activity and influence of parent material, vegetation, climate, time and topography. These influences have been termed the soil forming factors. The soil forming factors may be subdivided into active and passive factors. The passive factors, parent material and topography, serve as source material and conditioner, respectively, to which the active factors, vegetation and climate, supply the energy necessary for the processes to evolve. Time is also a necessary factor in that a period of time must elapse for the factors to approach dynamic equilibrium. Climate and vegetation are closely related since climate strongly influences the type of flora found

under a climax vegetation. The vegetative factor includes the influence of plants and animals which eventually form part of the soil organic matter. It is in the form of organic matter that plants and animals exhibit their greatest influence on soil genesis.

Early Russian Pedologists linked the process of podzolization with organic matter. Sprengel, Ramann, Kikuchaev, Sibertzev, Glinka, and others stressed the role of organic acids in the process of podzolization (45). This early work was completed in the latter half of the nineteenth and early twentieth century. The Russian knowledge of pedology was slow in reaching the West due to language barriers and internal strife within the country. Early soil scientists in America were interested in pedology primarily for practical applications. Not until Marbut's imaginative thinking introduced the Russian School of though did Americans visualize the soil as a three dimensional independent dynamic body.

More recently, soil organic matter has received increased attention in soil genesis and classification. Workers (20) have stressed vegetation as a necessary requirement for the formation of a soil. Duchaufour (29) has suggested that the type of organic matter is the principal factor in determining the genetic pathway followed in soil development. This has been stressed in his soil classification scheme based principally on genetic relationships as influenced primarily by the forms of organic matter.

In order to understand the role of organic matter in soil genesis, it is necessary to attain an insight into the structure, the physical and chemical properties, and the functions of the soil organic constituents. New techniques in the study of soils utilizing modern equipment and methods have assisted in replacing many postulations and theories by scientific facts in this field of pedology.

B. History of Soil Organic Matter Study

The early history of soil organic matter study has been thoroughly reviewed by Joffe (45). He stated that Berzelius in 1839 conducted systematic studies of organic materials. Berzelius isolated from springs and brooks crenic and apocrenic acids corresponding to the fulvic acids as recognized today. Mulder in the same year differentiated humus and similar substances according to their solubility in alkali. Mulder also determined the elemental composition of the so-called crenic and apocrenic acids. Schreiner and Shory in 1910 fractionated humic, crenic and apocrenic acids and isolated numerous free hydrocarbons and related compounds from these acids. Their main contribution to organic matter study was a recognition of the variability of organic matter composition as still recognized today. Khainskii in 1916 found that organic matter extracted from podzol soils was more soluble in acid solution than organic matter isolated from chernozems. This was suggested as evidence for the variations in the composition of organic matter

from different soils. Oden in 1919 recognized four component groups in soil organic matter:

1. Humus coal. This portion of organic matter is insoluble in alkali. It is also referred to as humin or ulmin.
2. Humic acid. This is the portion of organic matter which is soluble in alkali and insoluble in acids.
3. Hematomelanic acid. This organic matter portion is soluble in alkali and alcohol and insoluble in acids.
4. Fulvic acid. This portion of the organic matter is soluble in acids, alkali, alcohol and water.

Oden grouped the crenic and apocrenic acids of Berzelius in the fulvic acid group. These terms, although modified, are still used today to designate the different forms of soil organic matter.

A major contribution to organic matter study was the work of Waksman and Iyer (91) who in 1932 found that lignin exercised a depressive effect on the decomposition of proteins as a result of an interaction. The term "ligno-protein" was used to describe this stable material. In 1936 Waksman published his book "Humus" (89) which was recognized as an authority on soil organic matter for many years. More recently, Bremner (9, 10, 11) has written several thorough reviews on soil organic matter problems.

C. General Considerations of Soil Organic Matter

Joffe (45) has defined organic matter as any substance of organic origin, living or dead, found in the soil. It originates from two sources, original plant debris entering the soil, and microorganisms within the soil body itself. Plant residues, such as the celluloses, hemicelluloses, lignins, water soluble fractions, fats, oils, and proteins when added to the soil are immediately subjected to microbial decomposition. The soluble fraction, including the simple sugars, amino acids, aliphatic acids, are rapidly decomposed and act as a source of energy for the soil microflora. The more resistant portions of the plant residues, such as the lignins, fats and resins, remain in the soil for a greater length of time. Thus the added residues act as a substrate for the soil microbial populations and may be either completely mineralized to their inorganic state or decomposed, modified, and altered to such an extent that they may no longer be recognized as related to the original plant material.

Soil organic matter is comprised of a series of materials which range from undecayed plant and animal remains through ephemeral products of decomposition to relatively stable end products. These end products are amorphous brown to black materials bearing little similarity to the anatomical structure of the original material. This resistant material is generally considered to be humus. It is usually regarded

as that portion of the organic matter which has undergone decomposition to such an extent that the nature of the material from which it was derived cannot be determined.

Alexander (1) considered the following organic molecules present in humus; amino acids, purines, pyrimidines, aromatic molecules, uronic acids, amino sugars, pentose sugars, hexose sugars, sugar alcohols, methyl sugars, and aliphatic acids. This indicates the wide array and diversity of organic materials which are found in soils. These organic materials may be considered as existing in a dynamic state, continually under attack and being altered by subterranean flora and fauna.

Many workers (19, 45) have accepted the viewpoint of Oden and have recognized the four component parts of humus derived by empirical fractionation procedures. Workers in America, in contrast to European workers, generally group the hematomelanic acid with the fulvic acid portion. It should be noted that the components are not separate chemical entities. They are fractions obtained by empirical procedures and therefore may vary with slight variations in extraction procedures. Several workers (14, 47, 57, 90) have severely criticized the use of these terms.

The humus coal of soils sometimes referred to as humin or ulmin is primarily inert materials of high carbon content. Humic acids have received the most attention

of the empirical fractions and have been studied by many scientists. This fraction will be more fully discussed in Section E. The hematomelanic and fulvic acids, usually grouped together as fulvic acids, are relatively low molecular weight molecules of acidic character. Their structure has been postulated to be somewhat aromatic and very similar to humic acids except they exist in a less condensed state (49). Polysaccharides and polyuronides have been extracted from this fraction (35). According to Duchaufour (29), Tuirin separated the fulvic acids into two fractions:

1. Free fulvic acids. These fulvic acids are very soluble and their structure is thought to be phenolic in character similar to the structure of humic acids. They are characterized by acidic properties and are thought to attack mineral colloids and degrade clays. It is postulated that this fraction is responsible for initiating the podzolization process.
2. Fulvic acids attached to humic acids. These fulvic acids are extracted with the humic acids and are comprised of uronides and chain polysaccharides. They are less acidic than the free humic acids and are only slightly mobile in the soil.

The early workers of the 20th century in the field of soil organic matter realized the problem of establishing the structures of the components of the fractions. They did, however, believe that the structures could easily be worked out with their knowledge of chemistry and with the methods of analysis at their disposal. However, progress has been slow in this study and the problems of structure and behaviour are still unsolved. Perhaps in no other field of soil science does there occur such a divergence between reliable information and speculative assumptions. Some of the older concepts, such as the use of the terms humic and fulvic acids, still prevail in the literature and have not been replaced by more descriptive or meaningful terms.

D. Humus and Forest Litter

Types of Humus and Forest Litter

The classification of forest litter and soil humus has, in the past, been in a state of confusion. This confusion has arisen for a number of reasons such as the use of loose terms, inconsistent nomenclature, and lack of reliable information. For instance, terms such as raw humus, peat, acid humus, turf, duff, mor have all been used to designate essentially the same type of material. American soil scientists have paid little attention to soil humus constituents in contrast to European workers who place much more emphasis on the type and class of organic matter present in the soil. This is further substantiated by the fact

that in America only two principal groups of organic matter have been recognized, mull and mor. These are widely separated types of organic materials and numerous intergrades occur. These intergrades have not been fully appreciated by American soil scientists. In contrast, several groups and classes of organic matter have been recognized by European investigators.

Fraser (38) discusses humus in forested areas under two types: mull and mor. He suggests that the mull type of humus is found in soils common to forested areas that are relatively fertile, high in minerals, and characterized by a high base saturation. The forest litter in these areas contains a high mineral content. The soil organisms include vast numbers of earthworms which mix and churn the mineral rich organic constituents with the mineral portion of the soil. Under these conditions, each addition of litter is completely decomposed prior to the next year's leaf fall.

The mor type of organic matter is attributed to soils of low base status and relatively high acidity. The yearly addition of leaf litter is not mixed with the underlying mineral portion of the soil since there are very few earthworms among the soil organisms. The litter remains for more than one season before being humified.

With these forest conditions, 3 layers in the organic portion of the profile are usually discernable. They are: 1. Slightly altered litter. 2. A layer which

is partly decomposed. 3. A black humus layer consisting of the more stable end products of decomposition.

Lutz and Chandler (55) also suggest a classification of forest humus consisting of two main groups, mull and mor. The mull type, consisting of mixed organic and mineral matter with a gradual transition to the lower horizon, was subdivided into 5 types: coarse mull, medium mull, fine mull, firm mull, and twin mull. These types were separated mainly on the basis of structure. The mor type, consisting of a humus layer unincorporated with mineral material was subdivided into 5 types: matted mor, laminated mor, granular mor, greasy mor and fibrous mor. These latter types were classified on the basis of thickness of horizon development, degree of horizon development and structure. The above classification of Lutz and Chandler is applicable to forest humus layers of well-drained upland soils and is, therefore, of limited value.

Duchaufour (29) suggests a much more comprehensive classification scheme for soil humus based on two important criteria; 1, rate of mineralization, and 2, importance of biosynthesis which characterizes the humification, nature of decomposition products and the degree of association of the humus with mineral complexes. He recognized two broad categories of humus, aerobic and anaerobic. The anaerobic humus is found in soils saturated with water which results in poor aeration. These soils have few soil organisms to bring about organic matter decomposition and are therefore

characterized by an accumulation of organic matter. In this group Duchaufour placed three types:

1. Calcic peat, characterized by high calcium content and simple well decomposed organic materials which compose 70 to 75 per cent of the total materials.

2. Acid ologotrophic turf, characterized by a low base saturation, acidic pH, and a sphagnum moss vegetation.

3. Anmoor, characterized by a fluctuating water table, and an organic matter layer composed of an intimate mixture of clay minerals and decomposed transformed humus materials. These materials are well humified in calcic media.

The aerobic humus was subdivided by Duchaufour into four groups:

1. Calcic mull. The calcic mull group is characterized by a high calcium content, a well developed Ah horizon which is thick black or dark brown, and a granular stable structure. Bonding of the humic acids to the clays in these soils protects the humic acids from microbial attack. Burrowing animals bring about intensive mixing of the organic debris. The calcic mull type of humus may occur in calcimorphic soils and, in some cases, is similar to the "chernozemic Ah" humus. Subgroups recognized in this group are: forest calcic mull, calcic mull from calcarious turfs, humus of the steppes, and calcic mull-moder.

2. Mull forest. The mull forest types are found on soils high in calcium but with no free carbonates present in the profile. In contrast to the calcic mull, the calcium

activity may be low if mineralization is rapid. Since the mineralization progresses at a faster rate than in calcic mulls, transition to the underlying horizon may be progressive from the well developed Ah horizon. Some eluviation may then occur under extremely acid conditions. Subtypes of mull recognized are: mull types (earth mull), crypto mull, acid mull and hydromull.

3. Moder. The moder type of humus forms a transition between the mull and mor types. The Ah horizon is relatively thin, and the Ao horizon is thicker than the corresponding horizon found in the mull forest. Aggregation is poor. The acid moder was suggested to be a transition humus characteristic of forest soils in early stages of degradation as found in slightly leached Podzolic and Brown Podzolic soils. Subtypes of moder recognized are: acid moder, hydromorphic moder, alpine moder, and calcic moder.

4. Mor. The mor type of humus occurs under resinous forest cover on siliceous soils low in calcium content. The Ah horizon is thin or absent, but, if present, has a high carbon to nitrogen ratio. Decomposition of the organic debris is slow, forming a very thick Ao or H horizon and a sharp line of demarkation between the organic and inorganic horizons. The pH is less than 5 and often from 4 to 4.5. Subtypes of mor recognized are: granular mor, stratified or fibrous mor, hydromor and calcic mor.

<u>Organic Horizon Nomenclature</u>

Two types of organic horizon nomenclature are

generally being used. The National Soil Survey Committee of Canada (60) has recognized three organic horizons common to Canadian soils. They are:

L Horizon - An organic layer characterized by the accumulation of organic matter in which the original structures are discernable.

F Horizon - An organic layer characterized by accumulation of partly decomposed organic matter. The original structures are discernable with difficulty.

H Horizon - An organic layer characterized by an accumulation of decomposed organic matter in which the original structures are undefinable. This horizon nomenclature is largely employed in Europe. In contrast, the American Soil Survey Manual (86) designates organic horizons as Aoo and Ao horizons lying above the Ah horizon. The Aoo horizon is described as a surface horizon consisting of fresh litter generally less than one year of age. The Ao horizon is described as a surface horizon below the Aoo horizon and above the Ah horizon consisting of partly decomposed or matted plant remains.

In summary, it is evident that more stress has been placed on organic matter and forest litter in European countries than has been placed on this fraction of soils by investigators on this continent.

E. Humic Acid

Types of Humic Acids

Some European workers (29) have separated and classified several forms of humic acids. Various criteria, such as color, properties, and origin have been used as a basis for these separations. These workers suggest two types of humic acids based on origin.

1. Brown humic acids. The brown humic acids originate from the oxidation and transformation of lignin in a weakly acid media. They are brownish red in color and are characteristic of forest mull. They form complexes of low stability with clay minerals, are low in nitrogen, and are flocculated slowly by calcium.

2. Grey humic acids. These humic acids are derived by autolysis and microbial synthesis. They are dark in color, become intimately associated with clays, and are derived from the transformation of celluloses and hemicelluloses into quinone constituents which unite with acid amines. These humic acids are easily flocculated by calcium and are characteristic of chernozemic Ah horizons (calcic mulls).

According to Duchafour (29), Tiurin in 1951 and Ponomaryova in 1956 distinguished 3 types of humic acids.

1. Free humic acids. These humic acids are saturated with both calcium and hydrogen. They consist of comparatively small molecules weakly associated with clay minerals and result in poor soil structure. They are very

soluble in dilute alkali.

2. Humic acids bonded to clays by calcium. These humic acids are more condensed and are darker in color than the free humic acids. They cannot be dissolved by dilute alkali after decalcification.

3. Humic acids associated with clays by sesquioxide intermediaries. These humic acids are also highly polymerized and are associated with clays in elementary aggregates by exchangeable iron or aluminum. They cannot be extracted by dilute acid even after the removal of free sesquioxides.

The first group of Tiurin's humic acids closely resemble the brown humic acids of the European workers. The latter two groups of humic acids suggested by Tiurin, have condensed molecules and are intimately associated with clays similar to grey humic acids.

American soil scientists, in contrast to European Workers, have not subdivided humic acids. It is generally felt by workers on this continent that all humic acids are basically similar and that the fractions do not differ sufficiently to warrant their separation (14).

<u>Structure of Humic Acids</u>

The structure of soil organic matter and especially the structure of soil humic acids remains obscure. Their heterogeneous nature has defied all attempts of complete characterization.

The humic acid fraction in soils is usually regarded

as that portion of organic matter soluble in basic solutions and precipitated by the addition of mineral acids. This humic acid fraction comprises dark colored acidic complexes containg carboxyls, phenolics, methoxyls, acetyls, quinones, and other probable groups built on an aromatic nucleus. Humic acids generally contain from 1-5% nitrogen (35) and an ash content which is very difficult to lower less than 2-3% in podzols and 1% in chernozems (19,49). Burges (19) suggested that the ash content of humic acids are contaminants consisting of silica, calcium, sodium, iron and other elements. Humic acids are not only found in soils but are important components of coals, lignites and peats. Materials with properties similar to humic acids have also been isolated from leaves (65).

Humic acids occur primarily in the upper horizons in soils. More fulvic acids and less humic acids are usually found in organic matter isolated from lower depths (19). Burges (19) suggested that up to 75% of the organic materials of the litter layer of podzols may be humic material while chernozems may have about 40% of their organic material present as humic acids. He further suggested that although there are claims to the contrary, the humic acids are not distributed in any consistent pattern, either in the profile, or among different soils.

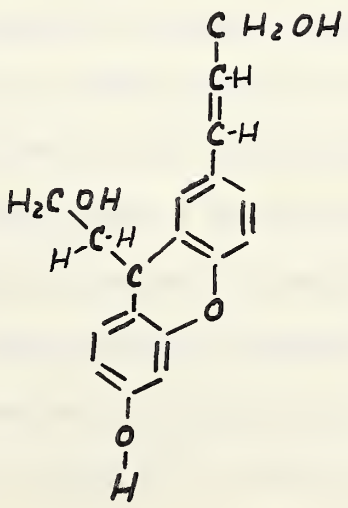
In the past, two theories on the origin of humic acids have been given wide support. The first theory is the ligno-protein concept of Waksman and Iyer (91) and the second

theory is that humic acids are the product of microbial synthesis. Supporters of the latter theory, according to Bremner (10), are Gel'tser, Kononova and Laatsch and Enders.

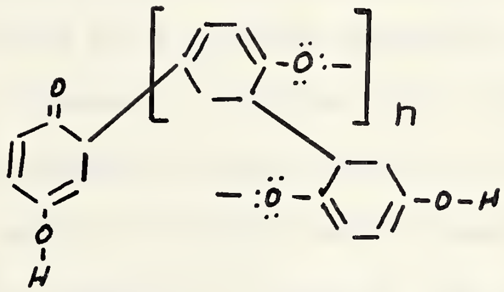
The ligno-protein concept suggested that humic acids are condensation products of resistant lignin and protein of plant origin. The synthesis concept suggested that humic acids are derived entirely from microbial synthesis. The plant materials are believed to be completely decomposed and resynthesized to humic acids by the soil organisms.

A more recent concept of humic acid formation is a combination of the above two theories. Workers (10, 46, 59) generally consider that humic acids originate from lignins of the plant residues added to the soil. These plant materials are modified and transformed by the soil flora and fauna.

Brauns (8) suggested that lignin is at least 75% phenyl propane building units with 2% phenolic hydroxyl. Methoxyl groups are meta to the propanol groups on the benzene ring in a structure as follows:



When lignin compounds enter the soil, the periphery groups undergo a series of hydrolysis, oxidation, and reduction reactions. As the methoxyl content of the lignin material decreases with decomposition, there is a corresponding increase in carboxyl and hydroxyl content. The aromatic rings rapidly polymerize (81) into a structure that could be similar to that shown by Zeichmann and Meyer (98).



The aromatic nature of humic acids has found popular support among numerous workers (46, 50, 57, 74, 95) although Savage and Stevenson (68) suggested humic acids contain insignificant amounts of stable aromatic structures. Wright & Snitzer (95) have shown that 49% of the carbon of humic acids isolated from an Ah horizon was aromatic and 48% aliphatic. Humic acids from a Bh horizon had almost identical aromatic carbon, however, a lower percentage of aliphatic carbon. The humic acids extracted from the Bh horizon contained more carbon in the form of carboxyl groups. Scheffer & Kickuth (69) found 47% of their humic acid sample

aromatic. Wright and Schnitzer (93) have shown that the aromatic benzene ring systems of humic acids are not completely fused since alkaline oxidation with $KMnO_4$ did not produce benzenehexacarboxylic (mellitic) acid. These aromatic structures form part of the organic fraction often termed "polyphenols" and are considered to be active in soil degradation.

Lewis and Broadbent (52) suggested that the acidity of humic acids arises from hydroxy quinones of altered lignin molecules. Farmer & Morrison (31) suggested that the acidity is due to carboxyl groups ortho to quinones. The acidity of humic acids is likely due to numerous functional groups with varying degrees of acidity and cannot be attributed to any particular group.

Steelink et al (79) suggested that humic acids extracted from podzol soils consist of an acid hydrolyzable portion and a non-acid hydrolyzable portion. They suggest that the hydrolyzable portion contains sugars, uronic acids amino acids, and soluble phenolics. The non-hydrolyzable portion contains the typical degradation products of lignins and tannins. This theory is in agreement with Misteriski and Loginov (57) who picture the humic acid particle as containing three zones. These are: 1. A nucleus, aromatic in nature. 2. A peripheral part, capable of undergoing hydrolysis and joined through active bonds to the nucleus. 3. A third zone consisting of mineral elements.

Scheffer and Kickuth (69, 70) found that humic acids contained an hydrolyzable periphery consisting mainly of celluloses, sugars and some amino acids; and a non-hydrolyzable nucleus. Of the total humic acids in their sample, they found 40% hydrolyzable, 5.6% ash, 6.1% ether soluble, and 47% non-hydrolyzable. Of the non-hydrolyzable nucleus, 30% was split off by reaction with hot nitric acid. This was considered to be the more active part of the nucleus of definite dimensions. The remaining part of the nucleus was postulated to be a coal-like material, deficient in active groups. Coulson et al (22) suggested that the sugars found in humic acids were nothing more than contaminants from the fulvic acid fraction, and are not an integral part of the humic acid molecule.

The molecular weights of humic acids still remain unknown. Wright et al (96) found average number molecular weight of organic matter to range from 47,000 to 53,800 as determined by osmometry. Wood et al (97) found a molecular weight of two humic acid samples extracted from coal to be 10,000. The molecular weights were determined cryoscopically with sulfolane as a solvent. It is questionable if this figure is applicable to soil humic acids.

In general, humic acids are considered to be organic molecules of high molecular weight with a distinctively acidic character. Although they have common structural characteristics, they are not completely identical. Humic acids are condensation and synthetic products of soil

biochemical reactions and are strikingly similar to polyhydroxy phenols which show a high degree of aromaticity. Although they are in themselves, very complex and diversified, it is strikingly evident that this diversification is relatively uniform throughout different soils.

Nitrogen and Humic Acids

The nitrogen present in humic acids appears to be strongly complexed. For example, Forsyth (35) found that nitrogen remained wholly attached to the humic acid molecule although the humic acids were subjected to methylation, acetylation and esterification procedures. He suggested that this nitrogen must be part of the molecular structure rather than present as a protein linked to the humic acids. Davis et al (24) suggested that humic acids are complexes of polyanions containing protein bound fragments of humic acids and nitrogen. The composition of the fragments may be determined but the mechanism by which they are associated with the humic acids remains obscure. Swaby (81) tested for proteins in humic acids and a negative result suggested the nitrogen was condensed as amino acids in the molecules. Brydon and Sowdon (17) suggested amino acids exist as a union between the organic matter and the hydrous oxides while Savage and Stevenson (68) suggested that the nitrogen of humic acids exists as bonds linking benzenoid rings. Sowden (76) could find no free amino groups in soil organic matter while other workers have identified amino acids in soil hydrolyzates (19, 80).

It would appear that nitrogen is an integral part of the humic acid molecule and may be isolated only by hydrolysis.

Physical Properties of Humic Acids

Although a mixture of humic acids and water appear to be in true solution, the mixture actually consists of finely dispersed colloids. Tyndal effect, osmotic effects, and other properties such as their inability to penetrate a semi-permeable membrane indicates a dispensoid.

Humic acids generally precipitate at a pH of 2.3 (57). The coagulating effect of calcium appears to be about the same over the pH range of 4 to 8; however, at a very high or a very low pH, increased amounts of calcium are necessary to cause flocculation (57). An increase in pH results in the retardation of the coagulating effect of iron and aluminum due to the decreased activity of these cations.

The presence of sulfates and especially the presence of phosphates appears to retard the coagulation of humic acids. There appears to be a mutual protective action between the phosphates and soluble humates. Calcium at a concentration sufficient to flocculate a solution of phosphorous or a suspension of humic acids would not flocculate a solution and a suspension of the phosphorus and humic acids together.

Misterski and Loginov (57) suggested that the gel or coagulated form of humic acids in soils is in dynamic equilibrium with humic acids in the dispersed phase.

This equilibrium is constantly being changed by numerous factors such as ion concentrations, pH, organic matter, decomposition, etc. The presence of phosphates in the soil favours the existence of humic acids in the dispersed phase.

Freezing causes the flocculation of a humus sol and may or may not be reversible. The fact that freezing destroys the colloidal properties of humic acids may be utilized as a technique for their purification (37). This freezing action likely has a definite stabilizing effect on soil colloids in the field.

Cations such as calcium, aluminum and iron have a flocculating effect upon humic acids while cations such as sodium, ammonium, potassium as well as anions such as the phosphates have a dispersive effect. The properties of humic acids are affected by freezing and thawing.

F. Polysaccharides and Polyuronides

Since polysaccharides are abundant constituents of plant animal tissues, they form an essential part of the soil organic matter. Polyuronides are considered important since their cementing ability results in better soil aggregation as has been claimed by several workers (66,84). These claims, however, have recently been disputed (42).

Forsyth (36) in 1950 found that polysaccharides extracted from diverse types of soils all yielded essentially

the same sugars on hydrolysis. He therefore suggested that they were a complex synthetic microbial product. Among the sugars identified by Forsyth were: galactose, galacturonic acid, glucose, mannose, arabinose, and xylose. Lynch et al (54) and Bernier (6) found various sugars in soils by chromatographic analyses. Among those identified were: galactose, glucose, mannose, arabinose, xylose, pentose, methylpentose, disaccharides, glucosamine, and mannitol.

Polyuronides are mainly glucuronic and galacturonic acids. These uronic acids may be described as sugar acids containing aldehyde and carboxylic groups. They differ from the other hexoses in that an hydroxyl group is replaced by the carboxyl group.

Duchaufour (29) pictured polysaccharides and polyuronides as forming a sequence in the soil. This sequence ranges from the more soluble substances found in the fulvic acid portion to large molecular constituents completely insoluble in alkali. He suggested that these molecules form a network which encloses clay colloids and humic acids, thus playing an important role in soil aggregation. Greenland et al (42) concluded that the effects of periodate treatment indicated polysaccharides and polyuronides were either not contributing at all to soil aggregation, or if they were, then their contribution was complementary to and dependent on other factors. Schnitzer and Delong (71), on the other hand, ascribed polysaccharides as being instrumental in causing mobilization of iron. Duchaufour (29) further

suggested that in biologically inactive soils, the organic matter decomposes to form numerous tannins and saccharides, ephemeric in character. Under certain conditions, these intermediary products of decomposition become resistant to decomposition and because of their solubility, play a significant role in iron mobilization. However, in biologically active soils, the soluble carbohydrates polymerize and progressive condensation of the molecules occurs. The polymerized carbohydrates become closely associated with the clay fraction. In their passage from soluble forms of low molecular weight to insoluble forms of higher molecular weights, the polysaccharides may produce cyclic or long chain structures. Duchaufour (29) suggested further, that the soluble polysaccharides and polyuronides may mobilize the clay and sesquioxides and thus enhance their movement down the soil profile. Polymerization of these carbohydrates into larger insoluble molecules in the lower solum results in the deposition of humus, clay and sesquioxides as horizons of accumulation.

It would appear that the role of polysaccharides and polyuronides in soil formation is conjectural, and the mechanisms and processes in which they are involved are speculative. Their role in the soil forming process is largely unknown.

G. Role of Organic Matter in Soil Degradation

Degradation of soils is generally associated with

the presence of forest cover in a relatively humid climate. Under certain environmental conditions, the decomposition of this organic matter is relatively rapid and little accumulation occurs. During the decomposition of the forest litter, soluble and colloidal organic constituents are released. Through the action of percolating waters, these organic constituents are washed downward into the mineral soil where they form soluble complexes with the clay minerals and sesquioxides. The complexes thus formed are relatively mobile, and it is in this manner that the organic constituents facilitate in the gradual removal of clays and sesquioxides from the upper most soil horizon. The mobilized clay and sesquioxides form a leached horizon of eluviation and an underlying horizon of accumulation.

The eluviation process may be physical or chemical, or a combination of the two processes (3). Physical movement of clay occurs when the colloidal complex is dispersed and the suspension moves down the profile to be coagulated by flocculating cations, such as calcium, iron or aluminum or by some other mechanism. Chemical movement occurs when the materials loose their mineral structure and move down the profile as sesquioxides to be deposited at a lower depth. The term podzolization has been used by North American soil scientists to refer to the two processes collectively, whereas European investigators use this term specifically to refer to the movement of clay by chemical means. Fridland (39) suggested the term "illimerization" for the process of clay migration without destruction. "Lessivage",

a term suggested by Duchaufour (29), has a similar meaning. Parfenova and Yarilova (62) criticized the use of these terms on the basis that the concept of podzol formation is wide, and includes simultaneous processes of clay transport and mineral decomposition. Pawluk (63) has shown that the Grey Wooded and Dark Grey Wooded soils of Alberta have formed largely by processes similar to "illimerization".

"Illimerization" or the physical movement of clay is ascribed to the peptization of the clay colloids by the dispersal actions of certain organic fractions. The mobilized clay is moved to lower depths in the profile where coagulation occurs due to the flocculation effect of increased concentrations of salts and/or microbial activity destroying the organic dispersing fraction. It has been suggested that several active constituents of the leachate of the decomposing forest litter are responsible for this mobilization. Bloomfield (7) stressed the importance of polyphenols and carboxylic acids. He isolated polysaccharides and substances with properties similar to polyphenols from aspen leaves. He considered these two constituents active in podzolization. He suggested that either one of these substances alone will bring about dispersion but it is necessary to have both present to bring about flocculation similar to that brought about by the original extract. Thorp _et al_ (83) showed the importance of organic acids such as oxalic and tannic acids in producing bleached A horizons and stronger coloured B horizons. They noted that extracts from a wide variety of

tree leaves resulted in varying amounts of clay mobilization when added to soil columns. Gallagher, according to Thorp et al, produced rapid reorganization of B horizon material by leaching with oxalic acid. Davis et al (23) suggested that the polyphenol responsible for degradation is derived from the washings of the growing leaves and not from the decomposing leaf litter as has been thought by most workers in this field. They suggested the amount of polyphenol in growing leaves varies inversely with the nutrient status of the soil. They further suggested that the fate of the polyphenol upon reaching the soil is determined by the soil reaction, the more acid the soil, the more stable the polyphenol. Coulson (22) reached similar conclusions.

Mechanisms suggested for the movement of sesquioxides involve metalo-organic complex formation (11, 71) although Debb (25) suggested that metals may also move as a humus-protected sol. Bremner et al (12) in 1946 was among the first to suggest that metals exist in soils as insoluble metalo-organic complexes. Beckwith (4) in 1955 supported this theory. Broadbent and Ott (15) suggested that the retention of cations by organic matter is not a simple case of salt formation since there is a difference between the values for mono- and di-valent cations. Other investigators (57, 77, 96) have all suggested strong inorganic-organic bonding.

Broadbent and Ott (15) found evidence to support the theory of chelate formation of $CuSO_4$ by organic matter.

The amount of $CuSO_4$ complexed was dependent on a number of factors including the cation involved, time of contact, pH, etc. He also suggested that the retention of polyvalent ions by humic acids may result from the formation of chelates or simple carboxylic salts. Chelate formation, therefore, may be one of the forms of metalo-organic complexes by which sesquioxides are mobilized in the podzolization process.

Pawluk (63) suggested the depth of Ae development in Grey Wooded soils is dependent upon the depth of penetration of the organic acids not having their hydrogen ions exchanged for aluminum or other ions. The penetrating organic acids exchange hydrogen ions for aluminum ions and other stabilizing ions on the clay complex. The replacement of hydrogen ions on the complex may have a partial dispersing effect on the clay particles as well as a dissolution effect. This would result in mild weathering of the clay minerals until recharge with aluminum or other ions moving from lattice positions to exchange positions occurs. Duchaufour (29) suggested the increase in degradation of a soil is accompanied by a general increase in the fulvic acid fraction.

In summary, the process of soil degradation results in the movement of clay particles and sesquioxides from the Ae horizon to points of accumulation in the lower solum. This movement is related to the decomposition products of organic matter. The decomposing organic products leach the mineral horizon, and the functional groups of the fulvic and humic acids combine with the sesquioxides and clay

minerals by forming complexes, thus rendering them more mobile and facilitating their downward movement.

H. Organic Matter Analyses

Polysaccharides and Polyuronides

Original determinations of carbohydrates were hardly more than mere approximations. For instance, polyuronides were determined by decarboxylation procedures and measurement of the carbon dioxide evolved. This method has been severely criticized (9) since numerous substances occur in soils which will evolve carbon dioxide under a range of analytical procedures. Waksman and Stevenson (92) determined the sugar content in soils by hydrolysis and subsequent measurement by their ability to reduce metal ions. This method was tedious and unreliable.

More recently, other methods have been suggested. Rennie et al (66) determined sugar content by purifying and drying the fulvic acid fraction; however, results were not reproducible. Brink et al (13) modified the anthrone colorimetric method suggested by Dreywood (26) for soil analysis. It has the advantage of being rapid and reproducible although not all polysaccharides are measured (13). Since this method appeared most suitable for polysaccharide analyses, it was used in this study.

In 1957 Lynch et al (53) modified the carbazole colorimetric method for determining polyuronides in plant

materials and applied it to soils. The presence of iron in soils interfered with color development and it was necessary to use two standard curves. Dormaar and Lynch (27) removed the iron by percolation of the extracts through a cation exchange resin. They also modified the extraction procedures. This method was used in this study since it appeared readily adaptable for the information desired.

Functional Groups

Brooks and Sternhell (16) in 1957 assessed several methods of determining oxygen functional groups. Carboxylic acids were previously determined by shaking samples in excess calcium acetate and determining the amount of acetic acid liberated by titration. Brooks and Sternhell (16) found that other alkali metals could be employed, and because of its greater solubility, suggested sodium acetate. They found that total acidity determinations carried out under nitrogen atmosphere were reproducible within 3 per cent. In comparing extractions under nitrogen to extractions under standard atmosphere, Wright and Schnitzer (94) found no change in the total acidity and methoxyl content. They noted a very small increase in carboxyl with an equivalent decrease in phenolic hydroxyl.

Infrared Analyses

Infrared analyses have been applied to soils in relatively recent times. Hunt <u>et al</u> (43) in 1950 published

the initial soil spectrum in relation to mineral identification. Durie and Murray (30) in 1957 were among the first to apply and assess the value of infrared analyses to the soil organic fraction. They found that for samples with 28% carbon or less, the spectra was dominated by strong mineral absorption. Since that time numerous workers have utilized infrared for studying soil organic constituents and changes occurring during humification (50), comparison of organic matter extractions (73), estimating aromaticity during oxidation (94) as well as changes occurring during organic-inorganic bonding (48).

Infrared analyses of soil organic and inorganic fractions have been used for a variety of projects and are well suited to study the functional groups of soil humic acids.

III. METHODS AND MATERIALS

A. Materials

Sample Areas

The areas from which the soil samples were collected lie in a region characterized by a sub-humid climate with an annual precipitation of approximately 18 inches and a mean annual temperature of approximately 36.5° F. The precipitation is received primarily during the summer months in which 55 per cent of the rain falls at rates less than 0.10 inches per day and 90 per cent at rates less than 0.50 inches per day. The mean annual humidity is 70 per cent.

The two soils selected for this study were of the Podzolic Order, Grey Wooded Great Group, and Dark Grey Wooded Sub Group. The first soil is typical of the Dark Grey Wooded while the second soil shows slight modifications of solonetzic processes as reflected by the morphological features.

Samples of the typical Dark Grey Wooded soil were collected from a site located at SW 3-53-2 W5 and will be referred to as Site 1 throughout this study. The soil is well drained and is formed on pitted deltaic parent material associated with glacial Lake Edmonton. It is characterized by an L horizon, 1 inch thick, mainly loose leaf litter, an F-H horizon, 2½ inches, darkish brown (10YR 3/3), fairly loose, an Ah horizon 10 inches, brownish black (10YR 3/2), friable, loam, an Ae horizon 10 inches, dark brown

(10YR 4/3), weak platy to granular, loam, a Bt horizon, 26 inches, yellowish brown, (10YR 5/4) weak subangular blocky, silty-clay loam, and a C horizon, pale brown (10YR 6/3), massive, silt loam. A more detailed description of the horizons sampled is given in Table 1. The site and profile are shown in Plate 1.

The sampling area was located in approximately 15 acres of native bush surrounded by cultivation. The samples were taken from two positions approximately 30 feet apart located near the centre of this virgin area. The native vegetation includes deciduous trees, approximately one half aspen poplar (_Populus tremuloides_ Mich.) and approximately one half balsam poplar (_Populus balsamifera_ L.). A smaller proportion of beaked willow (_Salix bebbiana_ Sarg. Var. _perrastrata_ (Rydb.) Schneid) was noted. The remainder of the vegetation noted were mostly shrubs and included:

1. Chokecherry (_Prunus virginiana_ L. var. _melancorpa_ (A. Nels.) Sarg.)
2. Saskatoon (_Amelanchier alnifolia_ Nutt.)
3. Common wild rose (_Rosa woodsii_ Lindl.)
4. Snowberry (_Symphoricarpos occidentalis_ Hook.)
5. Three-leaved Solomon's-seal (_Smilacina trifolia_ (L.) Desf.)
6. Long fruited anemone (_Anemone cylindrica_ A. Gray.)
7. Wild red raspberry (_Rubus melanolasius_ Focke.)
8. Golden rod (_Solidago pruinosa_ Greene.)
9. Northern reed grass (_Calamogrostis inexpansia_ A. Gray.)

TABLE 1

PROFILE DESCRIPTION OF SITE 1

Horizon	Depth Cm.	Color Dry	Color Moist	Structure	Consistency
L	6½-5	Darkish Brown (10YR 4/4)*	Dark Brown (10YR 3/3)	-	Loose
F-H	5-0	Dark Brown (10YR 3/3)	Lightish Black (10YR 2/2)	-	Loose
Ah	0-4	Brownish Black (10YR 3/2)	Lightish Black (10YR 2/2)	Granular	Friable
Ah	4-8	Brownish Black (10YR 3/2)	Lightish Black (10YR 2/2)	Granular	Friable
Ah	8-12	Light Black (10YR 4/2)	Brownish Black (10YR 3/2)	Granular	Friable
Ahe	12-16	Greyish Black (10YR 5/2)	Brownish Black (10YR 3/2)	Platy Granular	Friable to Firm
Ahe	16-20	Greyish Black (10YR 5/2)	Dark Brown (10YR 4/3)	Platy Granular	Friable to Firm
Aeh	20-24	Light Yellowish Brown (10YR 6/3)	Dark Brown (10YR 4/3)	Granular platy	Firm to Friable
Aeh	24-28	Light Yellowish Brown (10YR 6/3)	Dark Brown (10YR 4/3)	Granular platy	Firm to Friable
Ae	28-32	Light Yellowish Brown (10YR 6/3)	Dark Brown (10YR 4/3)	Platy	Firm
Ae	32-36	Very Light Yellow Brown (10YR 7/3)	Dark Brown (10YR 4/3)	Platy	Firm
Ae	36-40	Very Light Yellow Brown (10YR 7/3)	Dark Brown (10YR 4/3)	Platy	Firm

*Munsell colors.

PLATE 1. Site and Soil Profile at Site 1

The Dark Grey Wooded soil showing slight modifications of solonetzic processes as reflected by the morphological features is designated as Site 2. The sample site was located at NW 33-52-22 W4. The soil is well drained and is formed on glacial till parent material derived largely from the Edmonton geologic formation. It is characterized by an L horizon, 1 inch thick, loose leaf litter, an F-H horizon, 2½ inches, brownish black (10YR 3/2), fairly loose, an Ah horizon, 4 inches, light black (10YR 4/2) friable, loam, an Ae horizon, 14 inches, grey (10YR 6/1), platy, loam, Bt horizon, 8 inches, dark brown (10YR 4/4), clay loam, weakly columnar and a C horizon, dark greyish brown (10YR 4/2), clay loam, massive. A more detailed description of the horizons sampled is given in Table 2. The site and profile are shown in Plate 2.

The sample area was located in approximately 10 acres of native bush surrounded by cultivation. The samples were taken approximately 30 feet apart at the south east corner of the virgin area. The native vegetation was noted to be primarily aspen poplar (*Populus tremuloides* Mich.) balsam poplar (*Populus balsamifera* L.), and beaked willow (*Salix bebbiana* Sarg. var. *perrastrata* (Rydb.) Schneid.). The remaining trees and shrubs noted were:

1. Common wild rose (*Rosa woodsii* Lindl.)
2. Dogwood (*Cornus stolonifera* Mich.)
3. Snowberry (*Symphoricarpos occidentalis* Hook.)
4. Chokecherry (*Prunus virginiana L. var. melanocarpa* (A. Nels.) Sarge.)

TABLE 2

PROFILE DESCRIPTION OF SITE 2

Horizon	Depth Cm.	Color Dry	Color Wet	Structure	Consistency
L	7½-5	Darkish Brown (10YR 4/4)	Dark Brown (10YR 3/3)	-	Loose
F-H	5-0	Brownish Black (10YR 3/2)	Lightish Black (10YR 2/2)	-	Loose
Ah	0-4	Brownish Black (10YR 3/2)	Lightish Black (10YR 2/2)	Granular	Friable
Ah	4-8	Light Black (10YR 4/2)	Lightish Black (10YR 2/2)	Granular	Friable
Ahe	8-12	Greyish Black (10YR 5/2)	Brownish Black (10YR 3/2)	Platy granular	Friable to Firm
Ahe	12-16	Grey Black (10YR 6/2)	Brownish Black (10YR 3/2)	Platy granular	Friable to Firm
Aeh	16-20	Grey (10YR 6/1)	Brownish Black (10YR 3/2)	Granular platy	Firm to Friable
Aeh	20-24	Grey (10YR 6/1)	Light Black (10YR 4/1)	Granular platy	Firm to Friable
Ae	24-28	Grey (10YR 6/1)	Light Black (10YR 4/2)	Platy	Firm
Ae	28-32	Light Grey (10YR 7/1)	Dark Grey (10YR 5/1)	Platy	Firm
Ae	32-36	Light Grey (10YR 7/1)	Dark Grey (10YR 5/1)	Platy	Firm

PLATE 2. Site and Soil Profile at Site 2

B. Methods

Sampling and Laboratory Preparation

The L horizon, F-H horizon and layers every 4 centimeters through the mineral A horizon were sampled. Duplicate profiles were sampled at each site. At Site 1, 12 samples were collected from each profile and Site 2 was similarly sampled with a total of 11 samples from each profile.

The samples were air dried, hand-picked to remove all recognizable cellular organic materials, ground to pass through a 2 mm. sieve and stored in air-tight glass containers. Approximately 20 grams from each sample were hand ground with an agate mortar and pestle. These samples were also stored in air-tight glass containers and later used for carbon and nitrogen determinations.

Extraction of Humic Acids

Humic acids were extracted with 1N NaOH at a ratio of 4 solution to 1 soil for 16 hours at a temperature of 65° C. The supernatant liquid was decanted and centrifuged at 1200 r. p. m. for 20 minutes in an International centrifuge. This treatment removed the remaining inorganic materials from suspension. The humic acids were precipitated from the supernatant liquid by the addition of 5N HCl. After allowing approximately 12 hours for the humic acids to settle, the supernatant fulvic acids were partially removed by decantation and partially by centrifuging.

Quantitative Determination of Humic Acids

Fifty gram soil samples were used in the quantitative determination of humic acids. The extracted humic acids were partially purified by dialysis against running tap water for one week and further purified by freeze drying (37). The humic acids were dried, weighed, ashed and reweighed. Total humic acid content was determined by the loss in weight upon ignition.

Purification of Humic Acids

The humic acids were initially purified by the addition of distilled water and allowing the colloidal humic acids to settle. The supernatant liquid including salts was removed by decantation. This procedure was repeated several times until incipient dispersion was evident. This removed the major portion of the salts. The resulting extracts were made slightly alkaline in pH and further purified by exhaustive electrodialysis in a Mattson Type apparatus as shown in figure 1. The sample a was enclosed in a crock b with two semi-permeable membranes c, suspended by rubber corks d. Within the membranes was situated a copper cathode e, which also served as a drain and a platinum anode f. Distilled water entered at g, and removed the dialyzed impurities through h. When current was passed through the cell, the cathode assumed a negative charge and cations migrated through the semi-permeable membrane. The anode assumed a positive charge and the anions diffused

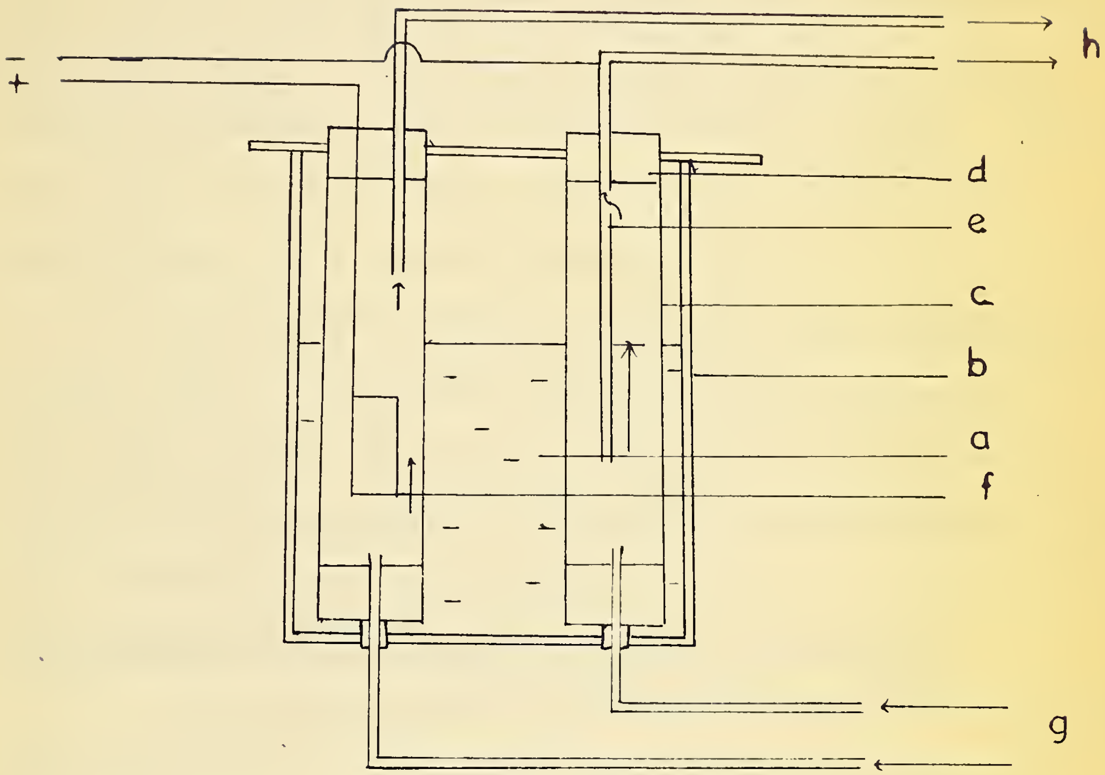


FIGURE 1. Electrodialysis Apparatus
For description see page 44

through the semi-permeable membrane. Flowing water removed the ions from the chambers. The dialysis was conducted at a potential of approximately 140 volts. At this potential the impure humic acids drew from 2 to 3 amps initially, depending on the rate of flow of the running water. The rate of water flow was adjusted to allow maximum removal of dialyzed salts with a minimum generation of heat. High temperatures resulted in the rupture of the membranes and subsequent loss of the sample. Nitrogen gas was bubbled through the humic acids to insure complete mixing. Practically all of the dialyzable material was removed after approximately 60 hours of electrodialysis.

After purification, the humic acids were dried on a water bath at 70° C and ground to a fine powder with a hand agate mortar and pestle. Functional groups, carbon content, nitrogen content, ash content, and infrared analyses were conducted on these prepared samples.

Determination of Polysaccharides and Polyuronides

The polyuronides and polysaccharides were determined by a colorimetric procedure. The transmittance was read on a Beckman Model B spectrophotometer at a wavelength of 625 millimicrons for the polysaccharides and 520 millimicrons for the polyuronides.

The carbohydrates were extracted by the method outlined by Dormaar and Lynch (27). Polysaccharides were determined by the method described by Brink et al (13) and the transmittance read against a water anthrone blank.

A standard curve complied well to Beer's law below 30 p.p.m. Dilutions were therefore adjusted to read within this range.

Polyuronides were determined by the method described by Lynch et al (53) as modified by Dormaar and Lynch (27). The latter workers suggested extracting the polyuronides with 3-4 N H_2SO_4. They further suggested that the optimum strength within this range should be determined for the samples being investigated. Therefore, samples of the 0-4 cm., 20-24 cm. and 36-40 cm. level of the A horizons from both sites were extracted with 3, 3.5 and 4 N H_2SO_4. The results are tabulated in Table 3.

TABLE 3

SPECTRAL ABSORBANCE OF SOIL SULFURIC ACID EXTRACTS FOR POLYURONIDE ANALYSES

H_2SO_4	Site 1			Site 2		
N	0-4	16-20	36-40	0-4	16-20	32-36
3.0	0.17	0.10	0.054	0.16	0.17	0.060
3.5	0.21	0.21	0.12	0.50	Green	Green
4.0	0.28	0.17	0.071	Slightly Green	Green	Green

The 4 N H_2SO_4 extracts showed considerable iron interference resulting in a green color rather than the desired characteristic cherry red of the polyuronide carbazole complex. The 3 N H_2SO_4 extracts showed the least absorbance and the 3.5 N H_2SO_4 extracts showed the greatest

absorbance in three cases out of four. The 3.5 N H_2SO_4 extracts indicated some iron interference at lower depths.

3.5 N H_2SO_4 was used in all subsequent extractions since maximum absorption, greatest accuracy and a minimum of iron interference was apparent at this strength.

Determination of Organic Carbon

The manometric method of VanSlyke and Folch (87), using the VanSlyke and Neill apparatus (88) was used for the determination of total carbon. The combustion mixture consisted of H_3PO_4, fuming H_2SO_4 (20 per cent SO_3), KIO_3 and $K_2Cr_2O_7$ as described by VanSlyke et al (89). Micro analyses were used for the carbon determinations of the soils, and macro analyses were utilized for the humic acids as suggested by VanSlyke et al (89).

Determination of Total Nitrogen

Total nitrogen was determined by the macro Kjeldahl method (2) except that the catalyst used was HgO (0.41 gm.), $CuSO_4$ (0.08 gm.) and K_2SO_4 (9.9 gm.) packaged in polythene bag and marketed commercially as Kel-pak*. The ammonia was collected in a 4 per cent H_3BO_3 solution as suggested by Meeker and Wagner (56), and titrated against standardized H_2SO_4.

Total nitrogen of the humic acids was determined by the micro Kjeldahl method described by St. Arnaud (78) on a sample of approximately 300 mgm. After digestion, the contents of the micro Kjeldahl were diluted to either

*D-C TAB No. 1, Digestion Catalyst Powders, Harshaw Chemical Co., Cleveland 6, Ohio.

200 or 250 ml. depending on the percentage of nitrogen expected. Five ml. aliquots were taken for the distillations. The ammonia was collected in 4 per cent H_3BO_3 solution and titrated against standardized H_2SO_4.

Functional Group Analyses of the Humic Acids

Total acidity and carboxyl groups were determined by the method described by Brooks and Sternhell (16). The total acidity was determined by baryta absorption ($Ba(OH)_2$) under a nitrogen atmosphere and the excess $Ba(OH)_2$ titrated with .1N HCl. A wetting agent (2 drops of 4% triton) was added to insure complete dispersal of the humic acid. Carboxyl groups were determined by treatment with an excess of sodium acetate and the released acetic acid titrated against .1N NaOH. Phenolic hydroxyl content was determined by the difference between the total acidity and carboxyl acidity.

Methoxyl groups were determined by a modified Zeisel technique (82). Constant heating, in this procedure, is necessary to insure refluxing of the digestion mixture; however, none of the sample should boil over into the collection tubes. This was achieved by emersing the combustion chamber in a glycerine bath kept at a constant temperature by means of a heater devised by wrapping a coil of electrically resistant wire around a 250 ml. beaker. The heater was insulated by wrapping asbestos on the outside of the container. The current was controlled by

the use of a continuously variable transformer.

Ash

Ash determinations on the humic acids were conducted by heating the samples for 1 hour at 700° C.

pH Measurements

The pH measurements were conducted on a saturated soil paste as outlined by Doughty (28), using a Beckman Model H-2 meter.

X-ray Analyses

X-ray analyses were determined with a Norelco high angle geiger counter X-ray diffractometer.

Infrared Analyses

A Perkin-Elmer Model 221G infrared spectroscope equipped with a prism-grating interchange was used for the infrared spectrum determinations. The spectra were determined as a Nujol mull in all cases.

Determination of Silica and Sesquioxides

Silica and total sesquioxides were determined gravimetrically. Iron was determined spectrometrically according to Shapiro and Brannock (75) and aluminum was calculated by difference.

Figure 2 shows a flow sheet of the fractionation procedures.

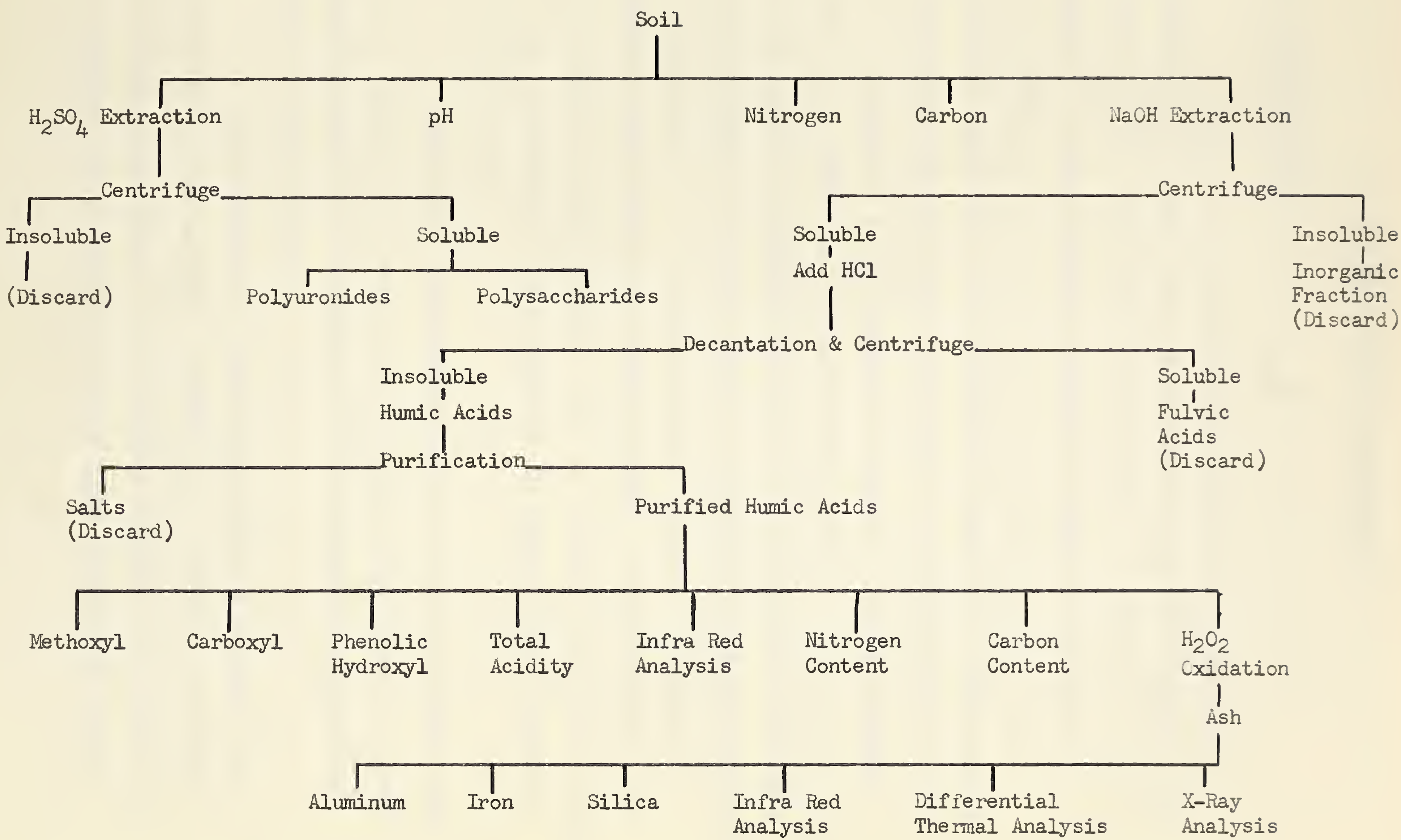


FIGURE 2. Flow Sheet

IV. RESULTS AND DISCUSSION

A. Introduction

One of the perplexing features of chernozemic degradation is that the eluviation process initiates at the bottom of the chernozemic Ah horizon. It would seem more logical for the leaching process to begin at the top of the chernozemic horizon since it is here that the soil particles which have been stabilized under the former grassland vegetation are first subjected to the degrading action of the effluents of the organic materials of the invading deciduous forest leaf litter decomposition. A second problem in this type of degradation arises from the fact that there is no evidence of organic matter accumulation in the horizons of illuviation. If decompositional products of organic materials are causing the mobilization of clay minerals in the horizons of eluviation, then it follows that there should be some evidence of these organic residues in the horizons of accumulation.

The above characteristics of the soils under study must be taken into account when considering organic matter characterization in relation to the degradation processes active in these soils.

The purpose of this study was to elucidate the mechanisms of chernozemic degradation. It was postulated that characterization of the organic matter constituents of the organic and A horizons of two Dark Grey Wooded soils

might aid in the clarification of the degradation process. Since the dynamic influence of humic acids in soils is well known (40), it was decided to focus attention on this fraction with minor emphasis on other constituents.

For the study of humic acids, corresponding soil samples taken from the duplicate profiles at each site were combined to reduce the number of infrared analyses that would be required. The resulting 12 samples from Site 1 and 11 samples from Site 2 were used for humic acid extractions and subsequent characterizations. Polysaccharide and polyuronide content were determined separately on all samples. The results of corresponding layers of the duplicate profiles from each site were averaged and the results are presented in this manner. This was done so that the results would be comparable to those obtained for the humic acids. All analytical data are reported as average of duplicates and on an oven-dry basis.

The Dark Grey Wooded soils used in this study are presently characterized by a deciduous forest type vegetation. This vegetation has resulted in a soil humus similar to moder and is therefore transitional between the mull and mor types. The organic matter accumulation from a year's vegetative growth is not completely humified before the next year's addition. The organic materials, however, are not mixed with the mineral materials as characteristic of the mull humus. This moder type appears to be superimposed over a relic chernozemic (calcic mull) humus formed under the grassland vegetation which was common to this region prior to forest

invasion.

B. Quantitative Determination of Humic Acids

Table 4 shows that the humic acid content is greater in the organic horizons and in the upper mineral Ah horizons than in the lower mineral horizons. The organic matter of these horizons have had insufficient time to decompose which may explain the high content of humic acids. The F-H horizons at both sites are low in humic acids while the underlying Ah horizons show a marked increase. Humification and mineralization may have released the humic acids in this horizon which have moved into the underlying Ah horizon.

Considering all samples analyzed, an average of 10.8% of the total soil carbon was found present as humic acid carbon. This is somewhat less than that reported by some European workers (19, 85).

C. Carbon Nitrogen Ratios and pH

The C/N ratios of the soil samples are given in Tables 5 and 6. The average C/N ratio of the Ah, Ahe and Aeh horizons at Site 1 is 11.5 and at Site 2, 12.6. Russell (67) suggested that a C/N ratio of 10 is characteristic of English soils while Buckman and Brady (18) suggested the C/N ratio of the furrow slice of arable soils commonly ranges from 8 to 15, the median being between 10 and 12. An average of the C/N ratio of the major Ah horizon of 13 Dark Grey Chernozemic and Dark Grey Wooded soils common to

TABLE 4

HUMIC ACID CONTENT

Site 1			Site 2		
Horizon	Depth Cm.	Humic Acids %	Horizon	Depth Cm.	Humic Acids %
L	6½-5	7.1	L	7½-5	12
F-H	5-0	2.6	F-H	5-0	1.6
Ah	0-4	3.3	Ah	0-4	2.7
Ah	4-8	1.5	Ah	4-8	1.4
Ah	8-12	0.85	Ahe	8-12	0.73
Ahe	12-16	0.36	Ahe	12-16	0.48
Ahe	16-20	0.30	Aeh	16-20	0.57
Aeh	20-24	0.21	Aeh	20-24	0.26
Aeh	24-28	0.18	Ae	24-28	0.42
Ae	28-32	0.045	Ae	28-32	0.36
Ae	32-36	0.17	Ae	32-36	0.38
Ae	36-40	0.14			

[illegible]

[illegible]

[illegible]			[illegible]		
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]		[illegible]	[illegible]	
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
			[illegible]	[illegible]	[illegible]

TABLE 5

VARIOUS PROPERTIES AND RATIOS OF THE SOILS AT SITE 1

Horizon	Depth Cm.	pH	Organic Carbon %	Nitrogen %	C/N	Polysaccharide Carbon of Total Carbon %	Polyuronide Carbon of Total Carbon %
L	6½-5	5.3	32.0	1.05	30.4*	6.8	1.1
F-H	5-0	5.7	19.3	1.06	16.6	5.4	3.7
Ah	0-4	6.3	10.3	1.80	12.9	11	7.6
Ah	4-8	6.2	5.8	0.47	12.3	8.6	1.9
Ah	8-12	6.4	3.8	0.29	13.1	6.6	1.6
Ahe	12-16	6.5	2.2	0.18	11.7	5.0	2.4
Ahe	16-20	6.2	2.3	0.12	10.7	5.4	3.3
Aeh	20-24	6.4	1.2	0.11	10.5	4.2	2.1
Aeh	24-28	6.2	1.0	0.11	9.6	5.4	2.2
Ae	28-32	6.1	0.98	0.098	9.9	4.0	2.1
Ae	32-36	6.3	0.92	0.092	9.9	3.3	2.1
Ae	36-40	5.6	0.84	0.084	9.9	5.0	2.0

* Ratios calculated prior to rounding of columns 3 and 4.

TABLE 6

VARIOUS PROPERTIES AND RATIOS OF THE SOILS AT SITE 2

Horizon	Depth Cm.	pH	Organic Carbon %	Nitrogen %	C/N	Polysaccharide Carbon of Total Carbon %	Polyuronide Carbon of Total Carbon %
L	7½-5	5.0	44.0	1.08	40.7*	8.6	1.8
F-H	5.0	5.6	19.3	1.28	15.1	9.6	4.2
Ah	0-4	5.5	14.1	0.86	16.5	12	4.6
Ah	4-8	5.7	5.4	0.42	13.1	6.4	1.2
Ahe	8-12	5.4	2.6	0.22	11.7	11	2.2
Ahe	12-16	5.4	2.4	0.20	11.8	7.8	1.8
Aeh	16-20	5.3	2.2	0.19	11.5	8.3	1.8
Aeh	20-24	5.2	2.0	0.18	11.3	6.4	1.7
Ae	24-28	5.2	1.8	0.16	11.4	5.2	1.5
Ae	28-32	5.1	1.6	0.14	11.6	5.4	1.6
Ae	32-36	4.9	1.2	0.11	11.0	6.2	2.0

* Ratios calculated prior to rounding of columns 3 and 4.

the forest tension zone of Alberta was 11.9*. These ratios are similar to those found in this study. High ratios are expected in the L and the F-H horizons since very little decomposition of the organic materials has taken place. The ratio would be expected to decrease with decomposition.

The pH of all horizons shown in Tables 5 and 6 indicate slightly acidic reactions. Site 2 is approximately one pH unit more acidic than Site 1.

D. Polysaccharides and Polyuronides

The anthrone method for determining polysaccharides is reproducible in the mineral horizons; however, with materials of higher carbohydrate content as found in the organic horizons, difficulties were experienced. Several determinations were conducted and in this manner the polysaccharide content of the organic horizons was obtained.

Figure 3 shows a rapid decrease in total polysaccharide content with depth at both sites. This may be expected since total organic carbon content also drops rapidly. Data presented in Tables 5 and 6 show slight decreases in the percentage polysaccharide carbon of total carbon in the lower layers of the eluviated Ae horizons, although this trend is not pronounced. Graveland and Lynch (41) found an increasing percentage of polysaccharide carbon of total carbon with increasing depth in the soil

---

*Unpublished data. Alberta Soil Survey.

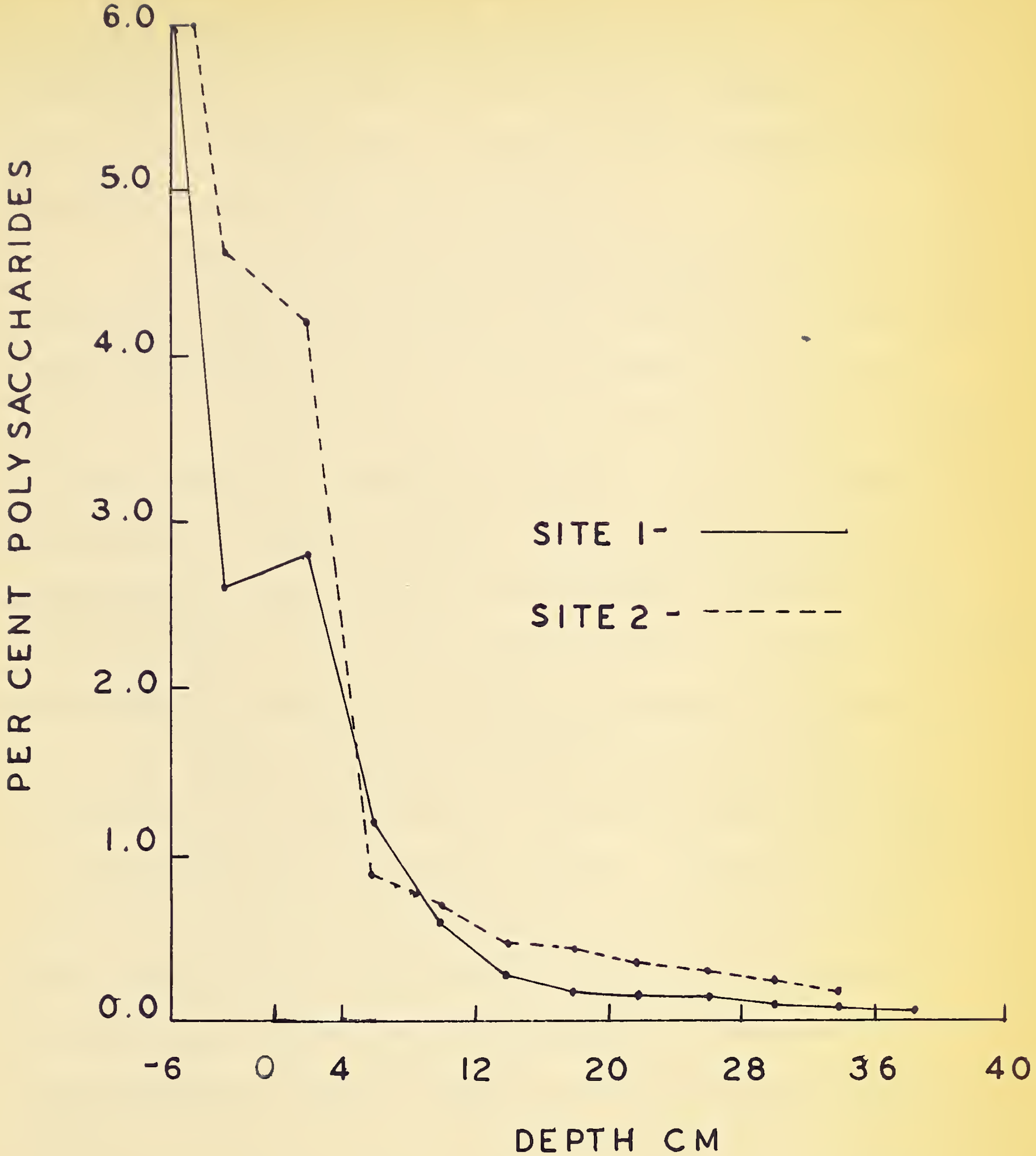


FIGURE 3. Polysaccharide Content in Relation to Soil Depth

profile. This increase with depth was attributed to the downward movement of soluble sugars. They were, however, comparing Ah, Bt and BC horizons and therefore comparing the sugar content of the soil solum as a unit rather than concentrating on one major soil horizon. Trends between major soil horizons may be different from trends between layers of a particular soil horizon.

The stabilizing influence of polyuronides on soil colloids is still questionable (42). The lower amounts of total polyuronides in the lower portions of the mineral A horizon in comparison to the polyuronides of the horizons near the surface is shown in Figure 4. This is likely due to a decrease in organic carbon. Tables 5 and 6 show the percentage of total carbon as polyuronide carbon is high in the organic horizons and the top four cm. of the Ah horizon. This ratio is relatively constant throughout the remaining A horizon layers. This may result from slower rates of decomposition in the horizons that have a lower percentage of total carbon as polyuronide carbon. However, this trend could also be an inherent characteristic of the organic matter of the moder type present in the surface horizons. Horizons with less carbon as polyuronide carbon could be characterized by organic matter of the chernozemic calcic type.

Figures 3 and 4 indicate that the soils from Site 2 contain greater amounts of both polysaccharides and polyuronides than the soils from Site 1. The average

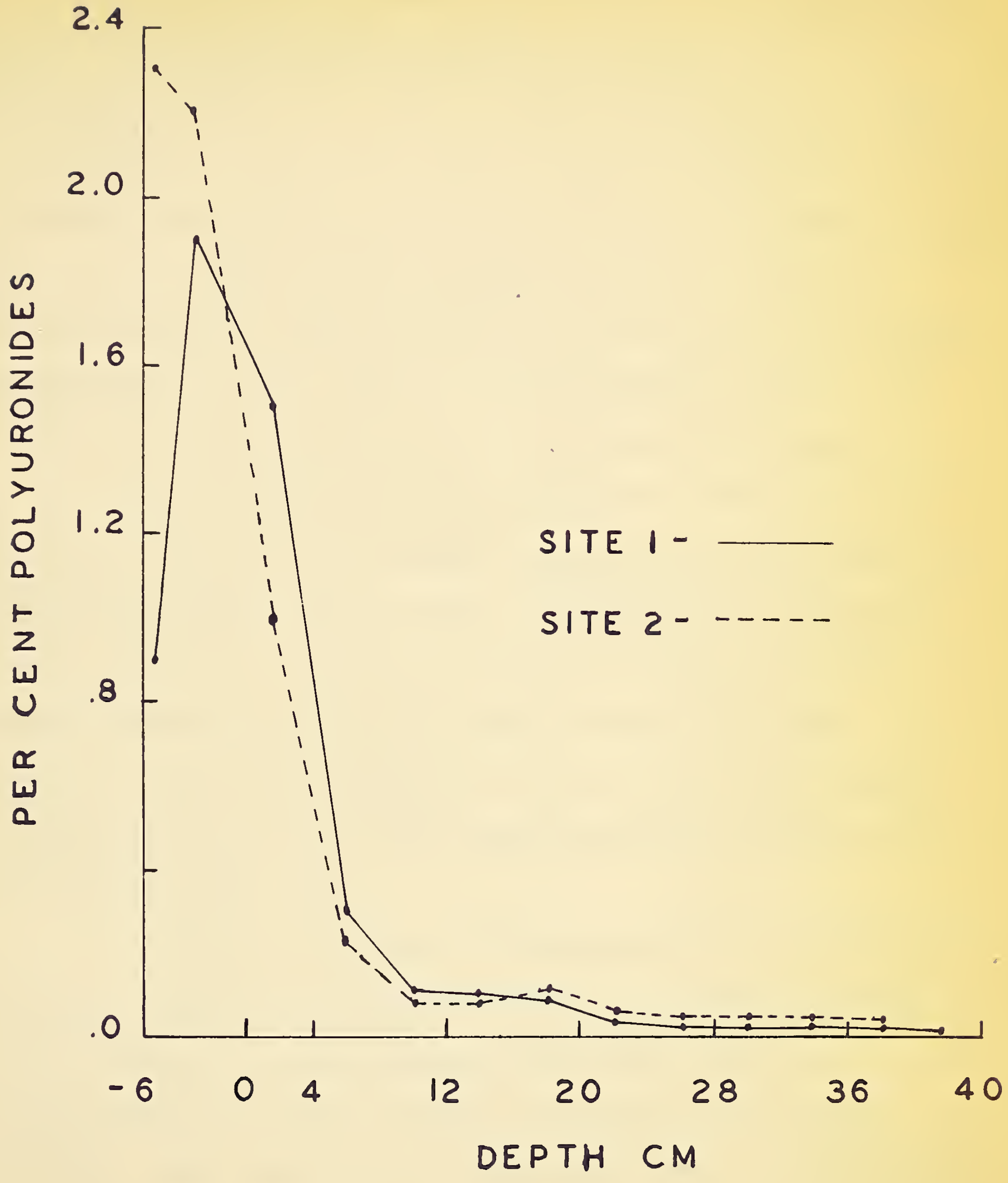


FIGURE 4. Polyuronide Content in Relation to Soil Depth

percentage of the total carbon as polysaccharide carbon was 7.9 for Site 2 and 5.9 for Site 1. Thus Site 2 also contains a greater percentage of the total carbon as polysaccharide carbon. The average percentage of the total carbon as polyuronide carbon was 2.21% for Site 2 and 2.67% for Site 1. It is noted here that the trend is reversed and Site 1 contains a greater percentage of the total carbon as polyuronide carbon than Site 2. Thus, Site 2 contains greater amounts of polysaccharides, greater amounts of polyuronides and greater percentages of total carbon as polysaccharide carbon, but a lower percentage total carbon as polyuronide carbon.

The average percentage of total carbon as polysaccharide carbon on all samples analyzed was 6.95% and the average percentage total carbon as polyuronide carbon was 2.44%. These two fractions contained 9.39% of the total carbon present in the soils. These results are more realistic than those reported by some of the older methods of determination such as the decarboxylation procedure for total polyuronides (9).

Although the total polysaccharides and polyuronides decreases rapidly with depth through the horizons studied at both sites, the percentage of the total carbon as polyuronide and polysaccharide carbon stays relatively constant. Thus the ratio of polyuronides and polysaccharides to total organic constituents show little change with depth. This would imply that polysaccharides and polyuronides are not taking an active part in the degradation processes of chernozemic Ah horizons.

E. Functional Group Analyses

Tables 7 and 8 indicate that the humic acids ranged from 0.14 to 0.63 with an average of 0.29 m.e. per gm. methoxyl at Site 1 and ranged from 0.11 to 0.51 with an average of 0.24 m.e. per gm. methoxyl at Site 2. The results show a slightly higher methoxyl content in the F-H horizon than in the L horizon. However, at both sites there is a pronounced decrease in methoxyl content with depth. This may be attributed to the fact that the humic acids extracted at greater depths have undergone further humifying and synthesizing transformations than the humic acids extracted at lesser depths.

Wright and Schnitzer (94) found 0.4 m.e. per gm. methoxyl content of humic acid extracts from an Ao horizon and 0.2 m.e. per gm. methoxyl content of humic acid extracts from the Bh horizon of a poorly drained podzol from Prince Edward Island. Since methoxyl content of organic matter decreases with decomposition (33), the high methoxyl content of these worker's extracts from the Ao horizon indicates a stage of decomposition between the F-H horizon and the Ah horizon of the soils used in this study. The content of 0.2 m.e. per gm. methoxyl of humic acid extract from the Bh horizon would indicate that the organic matter has not humified to the stage that the organic matter at the lower depths of the A horizon in this investigation. This may be due to the poorly drained conditions of the Podzol soil used in Schnitzer's study.

TABLE 7

FUNCTIONAL GROUP ANALYSES OF HUMIC ACIDS AT SITE 1*

Horizon	Depth Cm.	Methoxyl m.e./gm.	Total Acidity m.e./gm.	Carboxyl m.e./gm.	Phenolic Hydroxyl m.e./gm.
L	$6\frac{1}{2}$-5	0.52	6.2	3.0	3.3
F-H	5-0	0.63	6.0	2.8	3.3
Ah	0-4	0.43	7.0	4.8	2.2
Ah	4-8	0.36	7.0	4.1	2.8
Ah	8-12	0.26	6.5	3.1	3.4
Ahe	12-16	0.26	6.8	4.3	2.5
Ahe	16-20	0.21	8.4	5.3	3.0
Aeh	20-24	0.19	7.6	5.1	2.5
Aeh	24-28	0.16	7.6	4.6	2.9
Ae	28-32	0.16	9.3	4.9	4.4
Ae	32-36	0.14	9.4	4.2	5.2
Ae	36-40	0.21	9.3	4.7	4.6

*Ash-free Basis.

TABLE 8

FUNCTIONAL GROUP ANALYSES OF HUMIC ACIDS AT SITE 2*

Horizon	Depth Cm.	Methoxyl m.e./gm.	Total Acidity m.e./gm.	Carboxyl m.e./gm.	Phenolic Hydroxyl m.e./gm.
L	7½-5	0.37	6.8	3.1	3.7
F-H	5-0	0.51	5.3	3.6	1.7
Ah	0-4	0.34	6.7	3.0	3.7
Ah	4-8	0.30	8.0	4.2	3.8
Ahe	8-12	0.24	7.8	3.5	4.3
Ahe	12-16	0.18	7.5	3.8	3.7
Aeh	16-20	0.16	8.8	4.7	4.2
Aeh	20-24	0.11	8.1	3.5	4.6
Ae	24-28	0.11	8.9	5.2	3.8
Ae	28-32	0.11	8.1	3.8	4.3
Ae	32-36	0.18	9.6	4.1	5.5

*Ash-free Basis.

The slight increase in acidity of the humic acid extracts from the lower depths of the A horizon indicates greater activity of the humic acid molecules in the horizons of maximum eluviation. The similar results for the functional group analyses of the two sites would indicate that similar processes are involved.

F. Elemental Constituents and Ratios of Humic Acids

Table 9 shows a carbon content of 56% in the humic acids extracted from the L horizon and a marked decrease to 46% carbon in the humic acids extracted from the 36 to 40 cm. layer of the Ae horizon of the profiles sampled at Site 1. A similar trend in the carbon content in the humic acids extracted from the soils sampled at Site 2 may be noted. This decrease in carbon content may be attributed to the increased decomposition and transformations of the humic acids extracted from horizons lower in the profile. Increased humification may have resulted in further oxidation of the humic acids and subsequent formation of carboxyl and hydroxyl groups. The increased total acidity of the humic acids extracted from the lower depths further indicates the presence of greater numbers of functional groups.

The nitrogen content and C/N ratios of the humic acids show no trends throughout the horizons studied. It would appear that there is little alteration in the amount of nitrogen associated with humic acids as degradation proceeds. It is interesting to note, however, that although

TABLE 9

HUMIC ACID ANALYSES

SITE 1						SITE 2					
Horizon	Depth Cm.	Carbon %	Nitrogen %	C/N	Ash %	Horizon	Depth Cm.	Carbon %	Nitrogen %	C/N	Ash %
L	6½-5	56*	2.1*	26	0.088	L	7½-5	59*	2.2*	26	4.2
F-H	5-0	53	2.7	20	2.4	F-H	5-0	53	2.9	18	2.7
Ah	0-4	54	2.9	18	3.6	Ah	0-4	55	2.9	19	5 6
Ah	4-8	56	2.6	21	3.6	Ah	4-8	53	2.7	19	16
Ah	8-12	55	2.7	20	7.2	Ahe	8-12	54	2.7	20	7.9
Ahe	12-16	54	3.4	16	4.1	Ahe	12-16	53	2.8	19	14
Ahe	16-20	54	2.4	23	16	Aeh	16-20	56	2.6	22	7.9
Aeh	20-24	52	2.4	22	15	Aeh	20-24	54	2.6	21	8.1
Aeh	24-28	53	2.4	22	13	Ae	24-28	51	2.6	20	18
Ae	28-32	49	2.8	18	31	Ae	28-32	44	2.6	17	14
Ae	32-36	47	2.2	21	27	Ae	32-36	49	2.4	20	38
Ae	36-40	46	2.6	18	29						

*Ash-free Basis

the C/N ratios of the humic acids show slight variations between the layers, there is remarkable similarity in the ratio between humic acids extracted from the corresponding layers of the two sites. For instance, the L horizons of both sites show a high ratio of 26 which drops in the 0 to 4 cm. layer of the Ah horizon to 18 and 19 in Sites 1 and 2 respectively. In the 28 to 32 cm. layer of the Ae horizon, Site 1 shows a ratio of 18 while the ratio of Site 2 is 17 which further emphasizes the similarity. Wright and Schnitzer (95) reported 2.37% nitrogen content in humic acid extracts from the Ao horizon and 0.74% nitrogen in humic acid extracts from the Bh horizon of a podzol. The average nitrogen content of the humic acids extracted in this study was 2.6%.

G. Characterization of Humic Acid Ash

Table 9 shows that a relatively high ash content was found associated with the humic acid extracts from the lower layers of the A horizon. Since it appeared that ash was an integral part of the humic acids, this inorganic portion was studied further. Several samples were treated with hydrogen peroxide to remove the organic fraction. X-ray, infrared and differential thermal analyses were conducted on the residue, the analyses of which are shown in Figure 5.

The inorganic material was amorphous to X-ray diffraction prior to and after the hydrogen peroxide

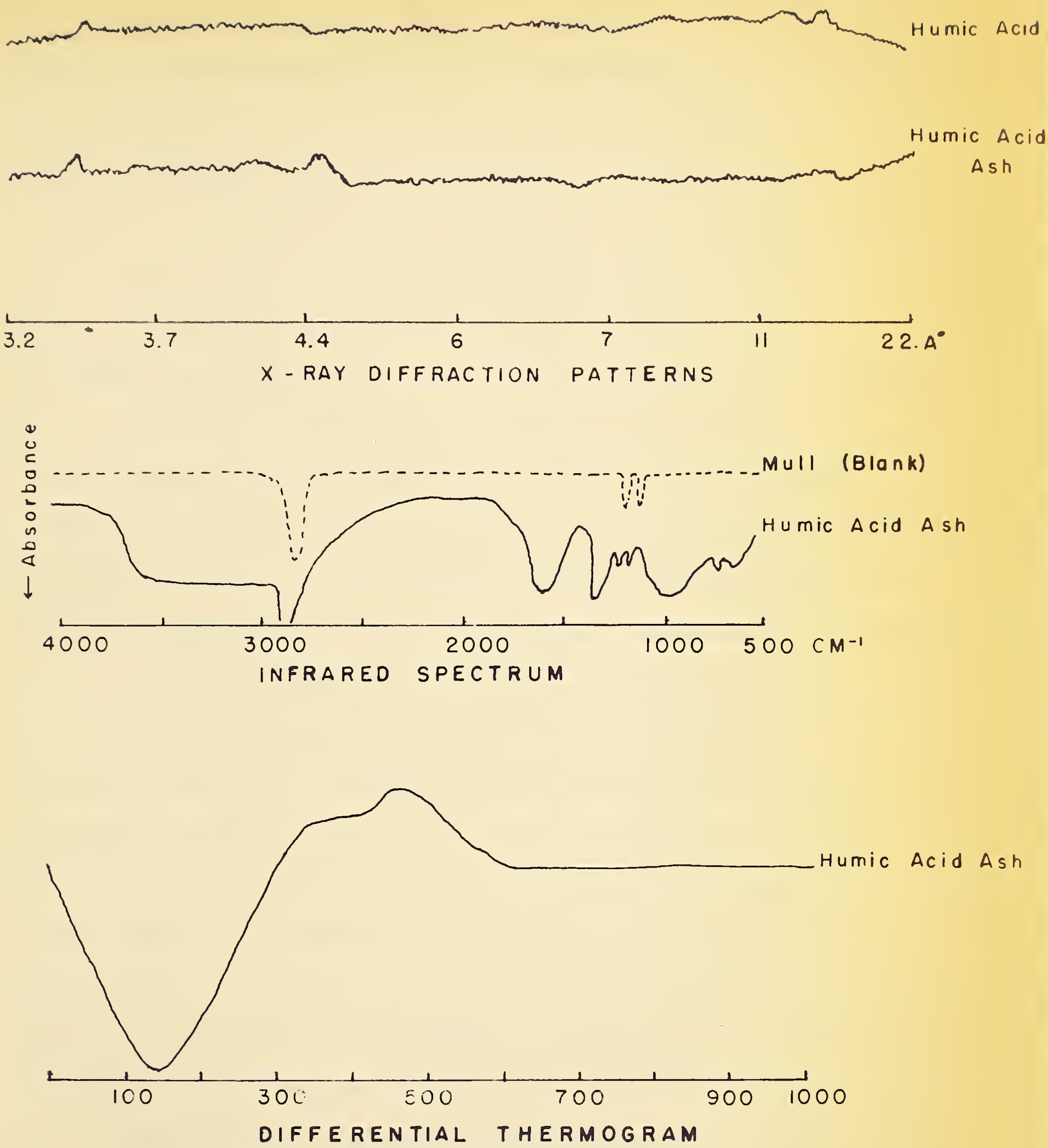


FIGURE 5. Some Analyses of the Humic Acid Residue

treatment. Similarily, no peaks in the differential thermogram are ascribed to crystalline decomposition. The endothermic peak at 150° C. may be ascribed to a large amount of adsorbed water while the exothermic peaks from 340 to 475° C. may be ascribed to residue of aromatic organic compounds. Chester et al (21) examined organic acids such as benzoic, malic, tartaric, citric, maleic and others, all of which showed exothermic reactions in this range. Since hydrogen peroxide does not oxidize some resistant waxes and oils, small amounts of resistant materials may be present in the residue. The close association of mineral humus complexes, however, cannot be excluded as an explanation for this organic residue. The closely associated minerals may exert a "protective" influence and prevent the organic residues from being oxidized by the hydrogen peroxide.

The wide band over the frequency range 3700 to 2500 cm.$^{-1}$ in the infrared spectrum can be attributed to the hydroxyls of the siliceous material and adsorbed water. The bands at 1638 and 1332 cm.$^{-1}$ can be attributed to the hydroxyl of combined water (43). This spectrum suggests a large amount of adsorbed water associated with the humic acid residue as was indicated by the differential thermogram. The wide band from 1100 to 900 cm.$^{-1}$ shown in the humic acid ash spectrum is likely due to silica. The width of this band may be indicative of its amorphous nature (5). Fieldes (32) suggested that amorphous silica also absorbs over a band from 840 to 760 cm.$^{-1}$ centered near 800 cm.$^{-1}$. The band in

this region of the ash spectrum may, therefore, substantiate the fact that the wide band from 1100 to 900 cm.$^{-1}$ shows that the silica is in an amorphous state.

Laboratory analysis of the ash residue after ignition at 700° C. showed that it contained 41.3% silica, 41.1% aluminum oxide and 8.6% ferric oxide.

It is concluded from the results discussed above that the material associated with the humic acids was largely silica and aluminum oxides, amorphous in nature, and contained a large amount of adsorbed water.

Several workers (4, 12, 15) have found close association of organic and inorganic soil materials as indicated by a high ash content of humic acids. Misterski and Loginov (57) suggested that the presence of greater than 10% ash often found in humic acids was evidence of humic acids existing as organo-mineral complexes. Schnitzer et al (72) found that the ash content of humic acids ranged from 20 to 30% after electrodialysis. They showed that the inorganic constituents associated with humic acids were predominantly illite. Other workers (17, 51, 59) found clay minerals associated with organic matter extracted by relatively mild procedures.

The close association of clay minerals with humic acids may be one method in which chernozemic Ah horizons are stabilized. As mentioned previously, the regions of Alberta where Dark Grey Chernozemic and Dark Grey Wooded soils are common were formerly characterized by a grassland vegetation.

The decomposition of the organic matter in the chernozemic Ah horizon resulted in large amounts of humic acids which, under the influence of bases, stabilized the soil clay mineral fraction. Upon forest invasion, the type of humus changed from one of chernozemic type to one of moder type. A large portion of the humic acids have, since forest invasion, been lost through mineralization especially in the lower layers of the chernozemic Ah horizons. Carbonates in these soils occur at such depths that they cannot be drawn up by the plants to the humus layers (44). This causes a weakly acidic character in these lower layers which is not neutralized by the addition of ash in the organic matter litter as in the case in the higher layers. Thus, the weakly acidic character of the lower Ah layers renders the clay minerals less intimately associated with humic acids and more susceptible to dispersion and migration to the underlying horizons of accumulation (44). This may partially explain why degradation is initiated in the lower layers of chernozemic Ah horizons in Dark Grey Chernozemic and Dark Grey Wooded soils.

The amounts of free silica and sesquioxides present in Ae horizons of Grey Wooded soils are relatively low (63). Consequently, mineral ash associated with humic acids would not be expected to exist in the amorphous form under field conditions. It follows, therefore, that the ash found associated with the humic acids in this study likely existed in the field as organo-clay complexes. Alkaline hydrolysis during the relatively harsh extraction procedure may have

caused the dissolution of these clay minerals. During the electrodialysis, the electronegative silica and the electropositive sesquioxides may have neutralized and co-precipitated with the humic acids.

H. Infrared Analyses

Figures 6 and 7 show the infrared spectrum of the purified humic acids. The first spectrum in Figure 6 is of Nujol, a high molecular weight paraffin hydrocarbon used as a "mulling agent" in the preparation of samples for infrared analyses. This spectrum corresponds to a blank. Since the Nujol absorbs only at the carbon hydrogen stretch and carbon hydrogen deformation frequencies and does not absorb at functional group frequencies, all samples were prepared by this technique. Initially, the spectra in general and assignments of frequencies will be discussed. This will be followed by a more detailed discussion of the spectra of the humic acids from each site.

The absorption band over the frequency range from 3700 to 2500 cm.$^{-1}$ in the spectra of the humic acids may be attributed to the hydroxyl stretch (5). Free hydroxyl groups absorb from 3700 to 3500 cm.$^{-1}$ and since hydrogen bonding is known to cause a decrease in frequency (5), this band may be expected to cover a wider range. The hydroxyl of the carboxyl group may contribute to this band over the 2700 to 2500 cm.$^{-1}$ range. The band at 1720 cm.$^{-1}$, which in some spectra resembles a shoulder, may be attributed to the

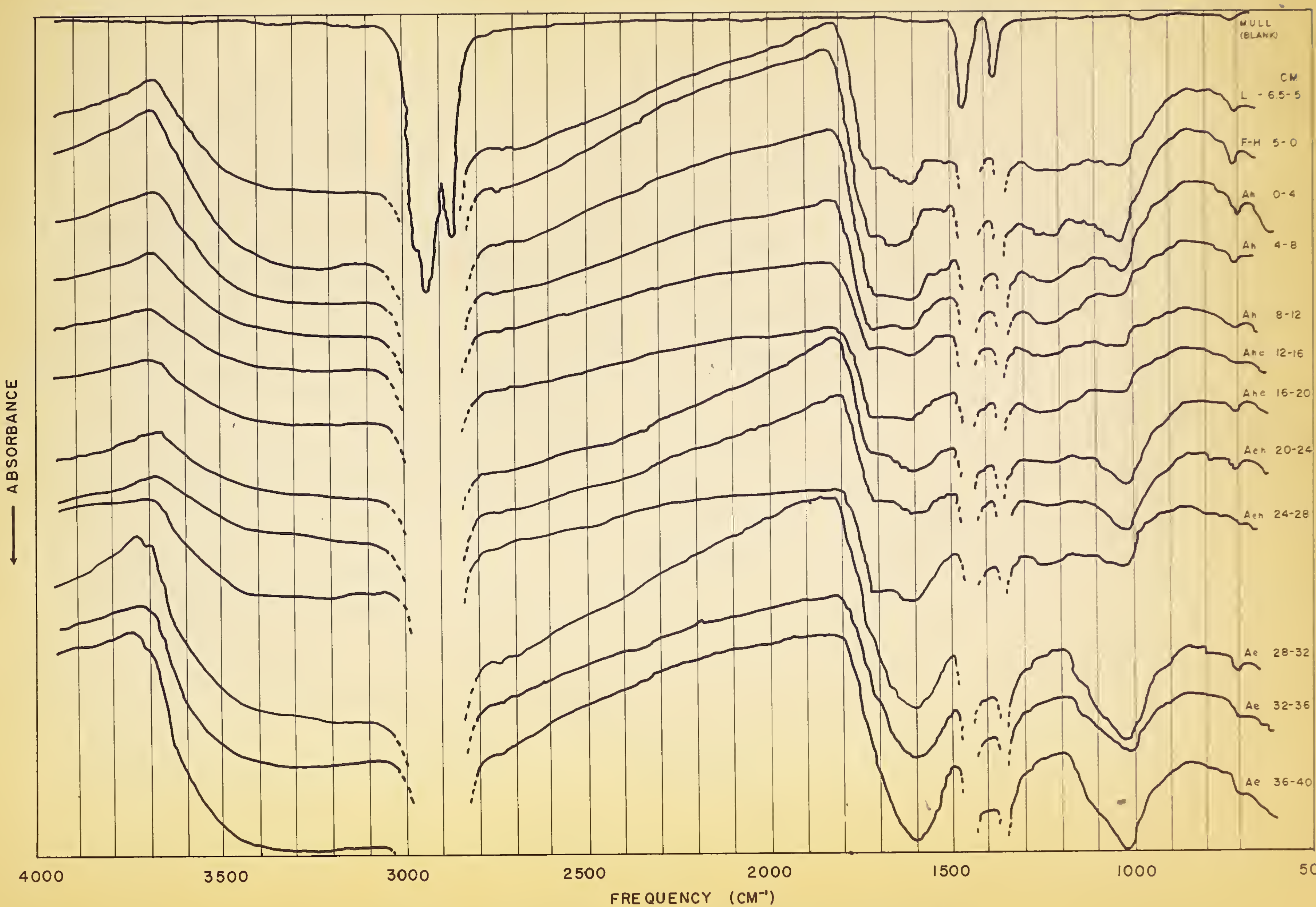


FIGURE 6. Infrared Spectra of Humic Acids from Site 1

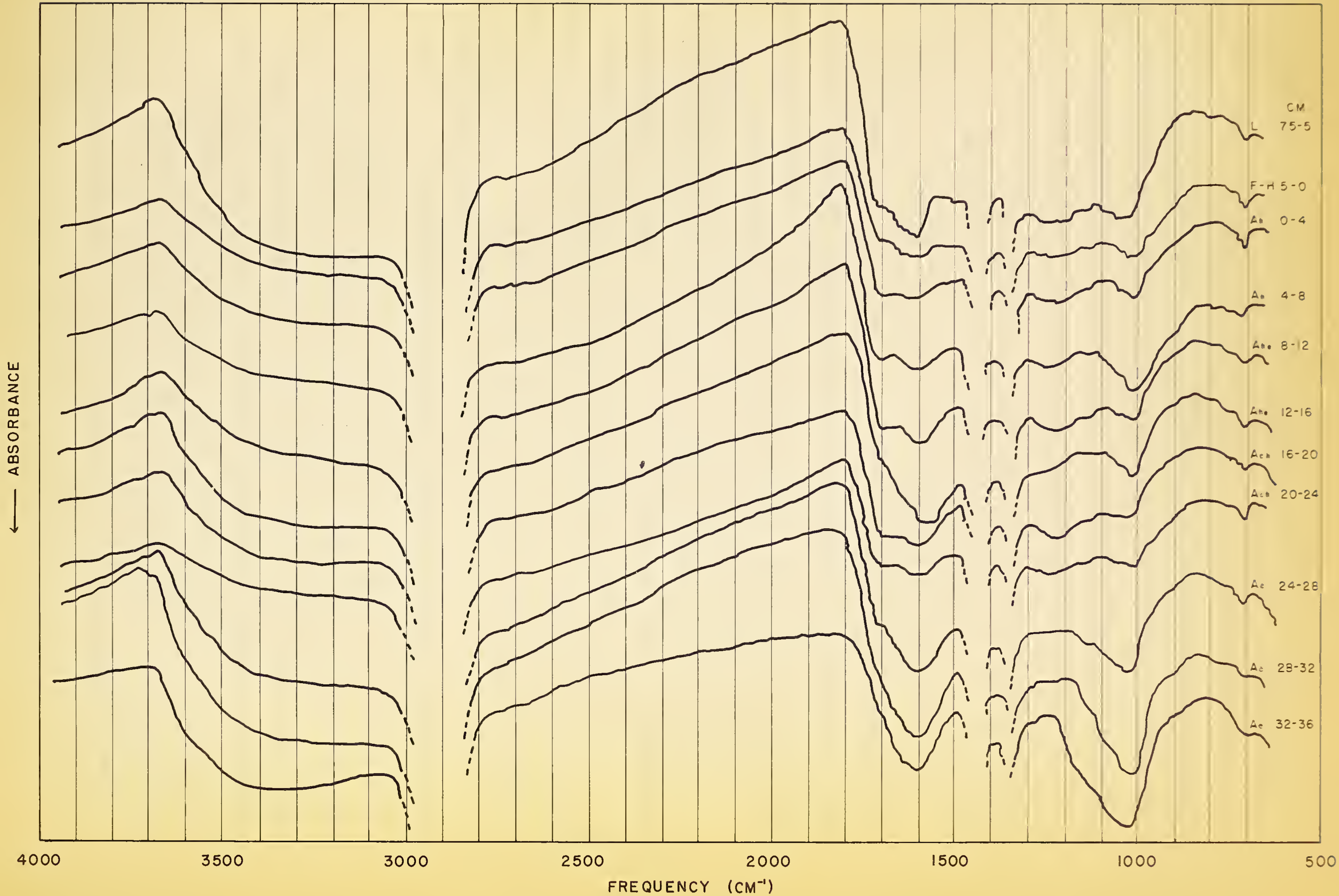


FIGURE 7. Infrared Spectra of Humic Acids from Site 2

carbonyl stretch of the carboxyl group (5). Absorption at 1637 cm.$^{-1}$ is attributed mainly to bonded hydroxyl of absorbed water (43, 48); however, quinones (mainly hydrogen bonded) are known to absorb in this region (5) and may also be contributing to this band. A band at 1025 cm.$^{-1}$ arises from the silica content of the humic acids (5).

The spectra of the humic acids extracted from the soil samples from Site 1 are shown in Figure 6. The strong band in the 3700 to 2500 cm.$^{-1}$ range in humic acids from the upper horizons can therefore be attributed to phenolic and alcoholic hydroxyl while the band in this region of the humic acids of the Ae horizon likely arises from the silica hydroxyl. The humic acids from the Ae horizons show a stronger 1637 cm.$^{-1}$ band, and a stronger silica band centered at 1025 cm.$^{-1}$. The band at 1720 cm.$^{-1}$ is attributed to the carbonyl stretch of the carboxyl group and is prominent in humic acids from horizons higher in the profile; however, it is somewhat masked in the Ae horizons by the high ash content.

The spectra of the humic acids extracted from the soil samples from Site 2 are shown in Figure 7. Trends in the spectra of these humic acids are similar to the trends noted in the humic acids from Site 1. Increased absorption in the band 3700 to 2500 cm.$^{-1}$ in the Ae horizon corresponds to high ash content with a similar increase in the band of the hydroxyl of absorbed water at 1637 cm.$^{-1}$ and the silica band at 1025 cm.$^{-1}$. The absorption of the carbonyl band

of the carboxyl group results in a shoulder at 1720 $cm.^{-1}$ in most of the humic acid extracts. Humic acids from the 12-16 cm. layer of the Ahe horizon contained 14.5% ash as indicated by Table 9. Stronger bands at the 1637 $cm.^{-1}$ and 1025 $cm.^{-1}$ frequencies in comparison to those of the neighboring layers are associated with the increase in ash of this sample. These bands are accentuated in the humic acid ash spectrum shown in Figure 5.

The infrared spectra of humic acids shown by Schnitzer *et al* (72) are similar to those presented here with the exception of a band at 1400 $cm.^{-1}$. This band was attributed by Schnitzer *et al* to the carboxylate ion. The presence of a carboxylate ion would not be expected in electrodialyzed humic acids since salts have been removed. In general, the infrared spectra shown in this study are characteristic of soil humic acids and confirm the results of the functional group analyses. In spectra of humic acids containing greater than 14% ash, absorption is dominated by that of the mineral content. The spectra of the humic acids extracted from the two sites are similar indicating the similarity of the humic acids extracted from these two soils.

V. SUMMARY AND CONCLUSIONS

The purpose of this project was to characterize the organic matter of two Dark Grey Wooded soils postulating that the results would assist in clarifying the degradation processes active in the genesis of soils common to the parkland area of Alberta. The characterizations were concentrated on the humic acid fraction with less emphasis on other organic constituents.

Characterizations of organic matter and soil humic acids have been carried out in the past; however, these characterizations have been of a general nature. This is believed to be the first instance that such a study has been carried out in detail on a particular soil type and a particular major soil horizon. This may, therefore, be regarded as a preliminary study in the relationship of soil organic matter to soil genesis in Alberta.

Some of the major conclusions of this study include:

1. A decrease in methoxyl content and a corresponding increase in total acidity of humic acids with depth through the mineral A horizon. These trends were similar at both sites.

2. A decrease in carbon content of the humic acids with depth. This may be a reflection of increased functional groups common to humic acids associated with horizons showing increased degradation.

3. A sharp decrease in polysaccharide and polyuronide content with depth. The per cent of the total carbon

present as polysaccharide and polyuronide carbon proved to be relatively constant throughout the horizons studied. This was shown to be true at both sites.

4. The amount of nitrogen associated with humic acids was relatively constant throughout the horizons studied.

5. A close association of soil organic matter and soil minerals. This was especially true at lower depths in the A horizon. The increased activity, as indicated by the increased total acidity, of the humic acids isolated from the samples of these lower depths may account for this close association. The humic acids characterized by fewer functional groups may have been less closely bonded to the clay minerals and rendered more susceptible to microbial attack. They may, therefore, have been lost through mineralization.

6. The mineral material associated with the humic acids proved to be amorphous although it was thought that they existed in the field as organo-mineral complexes. The mineral material consisted primarily of silica and sesquioxides.

The Dark Grey Wooded soil which was designated as Site 2 in this study was characterized by a columnar structure in the B horizon, indicative of solonetzic features. This soil, as previously mentioned, may be regarded as an intergrade between the Dark Grey Wooded and the Eluviated Chernozem. From this study, it is concluded that similar processes are presently active in the degradation of the chernozemic Ah horizons of these soils as are active in the degradation of the chernozemic Ah horizons in the typical Dark Grey Wooded soils.

The results of this study have somewhat clarified the role of organic matter in the degradation of chernozemic Ah horizons in Dark Grey Chernozemic and Dark Grey Wooded soils. For instance, it would appear that humic acids are not responsible for the mobilization of clay minerals in the active degradation processes. The role of humic acids in the genesis of these soils may be primarily as a stabilizing agent. The results further suggest that polysaccharides and polyuronides are not active in the degradation of chernozemic Ah horizons.

VI. BIBLIOGRAPHY

1. Alexander, M. Introduction to soil microbiology. John Wiley and Sons, Inc. New York. 1961.

2. Association of Official Agricultural Chemists. Official methods of analyses. 8th ed., Washington, D.C. 1955.

3. Bartelli, L. F. and R. T. Odell. Laboratory studies and genesis of clay-enriched horizon in the lowest part of the solum of some Brunizem and Grey Brown Podzolic soils in Illinois. Soil Sci. Soc. Amer. Proc. 24:390-395. 1960.

4. Beckwith, R. S. Metal complexes in soils. Aust. J. Agric. Res. 6:685-698. 1955.

5. Bellamy, L. J. The infra-red spectra of complex molecules. John Wiley and Sons, Inc. New York. 1958.

6. Bernier, B. Characterization of polysaccharides isolated from forest soils. Biochem. J. 10:590. 1958.

7. Bloomfield, C. The possible significance of polyphenols in soil formation. J. Sci. Food Agric. 8:389-392. 1957.

8. Brauns, F. E. The chemistry of lignin. Academic Press, Inc., New York. 1952.

9. Bremner, J. M. A review of recent work on soil organic matter. Part I. J. Soil Sci. 2:67-82. 1951.

10. Bremner, J. M. A review of recent work on soil organic matter. Part II. J. Soil Sci. 5:214-232. 1954.

11. Bremner, J. M. Some soil organic matter problems. Soils and Fertilizers XIX:115-123. 1956.

12. Bremner, J. M., S. G. Heintze, P. J. G. Mann, and H. Lees. Metallo-organic complexes in soils. Nature 158:790-791. 1946.

13. Brink, R. H. Jr., P. Dubach and D. L. Lynch. Measurement of carbohydrates in soil hydrolyzates with anthrone. Soil Sci. 89:157-166. 1960.

14. Broadbent, F. E. Basic problems in organic matter transformation. Soil Sci. 79:107-114. 1955.

15. Broadbent, F. E. and J. B. Ott. Soil organic matter-metal complexes. 1. Factors affecting retention of various cations. Soil Sci. 83:419-427. 1957.

16. Brooks, J. D. and S. Sternhell. I. Oxygen containing functional groups in victorian brown coals. Austr. J. Appl. Sci. 8:206-221. 1957.

17. Brydon, J. E. and F. J. Sowdon. A study of the clay-humus complexes of a chernozemic and a podzol soil. Can. J. Soil Sci. 39:136-143. 1959.

18. Buckman, H. O. and N. C. Brady. The nature and properties of soils. 6th ed. The Macmillan Co. New York. 1960.

19. Burges, A. The nature and distribution of humic acid. Sci. Proc. Royal Dublin Soc. Series A. 1:53-58. 1960.

20. Byers, H. G., C. E. Kellogg, M. S. Anderson and J. Thorp. Formation of soil. U. S. Department of Agriculture Yearbook of Agriculture. "Soils and Men" U. S. Government Printing Office, Washington, D.C. 1938.

21. Chester, G., O. N. Allen, and O. J. Ottoe. Differential thermograms of selected organic acids and derivatives. Soil Sci. Soc. Amer. Proc. 23:454-457. 1959.

22. Coulson, C. B., R. I. Davis and E. J. A. Khan. Humic investigations. 3. Studies on the chemical properties of certain humic acid preparations. Soil Sci. 88:191-195. 1959.

23. Davies, R. I., C. B. Coulson, and D. A. Lewis. Polyphenols in soil profile development. Sci. Proc. Royal Dublin Soc. Series A. 1:183-189. 1960.

24. Davies, R. I., C. B. Coulson and C. Luna. Humic acid investigations. Chem. Ind. 1957:1544.

25. Debb, B. C. The movement and precipitation of iron oxides in podzol soils. J. Soil Sci. 1:112-122. 1950.

26. Dreywood, R. Quantitative test for carbohydrate material. Ind. Eng. Chem. Anal. Ed. 18:499.

27. Dormaar, J. F., and D. L. Lynch. Amendments to the determination of polyuronides in soils with carbazole. Soil Sci. Soc. Amer. Proc. 1962. In Press

28. Doughty, J. L. The advantages of a soil paste for routine pH determinations. Soil Sci. 22:135-138. 1941.

29. Duchaufour, P. Precis de pedologie. Masson & C E'diteurs, 120 Boulevard, Saint-Germain, Paris. 1960.

30. Durie, R. A. and A. Murray. A study of the infra-red absorption spectra of soils. Australian J. Applied Sci. 8:335-338. 1957.

31. Farmer, V. C. and R. I. Morrison. Chemical and infrared studies on phragmites peat and its humic acid. Sci. Proc. Royal Dublin Soc. Series A. 1:85-104. 1960.

32. Fieldes, M. Clay mineralogy of New Zealand soils. Part II. Allophane and related mineral coloids. New Zealand J. Sci. and Techol. B. 37:336-350. 1955.

33. Flaig, W. Comparative chemical investigations on natural humic compounds and their model substances. Sci. Proc. Royal Dublin Soc. Series A. 1:149-162. 1960.

34. Forsyth, W. G. C. Studies on the more soluble complexes of soil organic matter. I. Method of fractionation. Biochem. J. 41:176-181. 1947.

35. Forsyth, W. G. C. The characterization of the humic complexes of soil organic matter. J. Agr. Sci. 37:132-138. 1947.

36. Forsyth, W. G. C. Studies on the more soluble complexes of soil organic matter. II. The composition of the soluble polysaccharide fraction. Biochem. J. 46:141-146. 1950.

37. Forsyth, W. G. C. and G. K. Frazer. Freezing as an aid in the drying and purification of humus and allied material. Nature 160:607. 1947.

38. Fraser, G. K. Soil organic matter. "Chemistry of the soil" A. C. S. Monograph 126. Ed. by Firman E. Bear. Reinhold Publ. Corp. New York. 1958.

39. Fridland, V. M. Podzolization and Illimerization. Pochvovedenie 1958, No. 1:24-32.

40. Gillman, W. S. A study on the chemical nature of humic acid. Soil Sci. 49:433-453. 1940.

41. Graveland, D. N. and D. L. Lynch. Distribution of uronides and polysaccharides in the profiles of a soil catena. Soil Sci. 91:162-165. 1961.

42. Greenland, D. J., G. R. Lindstrom, and J. P. Quirk. Role of polysaccharides in stabilization of natural soil aggregates. Nature 4795:1283-1284. 1961.

43. Hunt, J. M., M. P. Wisherd, and B. C. Lawrence. Infrared absorption spectra of minerals and other inorganic compounds. Anal. Chem. 22:1478-1497. 1950.

44. Ivanova, Y. N. and N. N. Rozov. An attempt at a systematic classification of the soils in the steppe zone of the U. S. S. R. Pochvovedenie 1958. No. 12:1349-1358.

45. Joffe, J. S. Pedology. Pedology Publications, New Brunswick, New Jersey. 2nd ed. 1949.

46. Johnston, H. H. Soil organic matter. II. Studies of the origin and chemical structure of soil humic acids. Soil Sci. Soc. Amer. Proc. 25:32-35. 1961.

47. Kaurichev, I. S., Y. A. Fedorov and I. A. Shnabel'. Separation of humic acids by the continuous paper-electrophoresis method. Pochvovedenie 1960. No. 10:1050-1053.

48. Kohl, R. A. and S. A. Taylor. Hydrogen bonding between the carbonyl group and Wyoming bentonite. Soil Sci. 9:223-227. 1961.

49. Kononova, M. M., and N. P. Bel'chikova. Investigation of the nature of soil humus substances by fractionation. Pochvovedenie 1960. No. 11:1149-1155.

50. Kumada, K. and K. Aizawa. The infra-red spectra of humic acids. Soil and Plant Food. 3:152-159. 1958.

51. Kumada, K. and K. Aizawa. The infrared absorption spectra of soil components. Soil and Plant Food. 4:181-188. 1959.

52. Lewis, T. E. and F. E. Broadbent. Soil organic matter-metal complexes. 4. Nature and properties of exchange sites. Soil Sci. 91:393-399. 1961.

53. Lynch, D. L., E. E. Hearns, and L. J. Cotnoir Jr. The determination of polyuronides in soils with carbazole. Soil Sci. Soc. Amer. Proc. 21:160-162. 1957.

54. Lynch, D. L., L. M. Wright and H. O. Olney. Qualitative and quantitative chromatographic analyses of the carbohydrate constituents of the acid-insoluble fraction of soil organic matter. Soil Sci. 84:405-411. 1957.

55. Lutz, H. J. and R. F. Chandler. Forest soils. John Wiley and Sons, Inc. New York. 1949.

56. Meeker, E. W. and W. C. Wagner. Titration of ammonia in presence of boric acid. Ind. Eng. Chem. Anal. Ed. 5:396-398. 1933.

57. Misterski, W. and W. Loginov. A study of some physicochemical properties of humic acids. Pochvovedenie 1959. No. 2:170-180.

58. Moss, E. H. The vegetation of Alberta. Bot. Rev. 21:467-493. 1955.

59. Murphy, D. and A. W. Moore. A possible structural basis of natural humic acid. Sci. Proc. Royal Dublin Soc. Series A 1:191-195. 1960.

60. National Soil Survey Committee of Canada. Proceedings of the fourth national meeting. 1960.

61. Newton, J. D., W. Odynsky and T. W. Peters. Some characteristic bleached horizon soils of Alberta, Canada. Trans. 5th Intern. Congr. Soil Sci. Vol. IV:286-295. 1954.

62. Parfenova, Y. I. and Y. Yarilova. The problem of lessivage and podzolization. Pochvovedenie 1960. No. 9:913-925.

63. Pawluk, S. Characteristics of Grey Wooded soils developed from glacial till deposits in the Peace River districts and north central regions of Alberta. Trans. 7th Intern. Congr. Soil Sci. Vol. IV:314-322. 1960.

64. Pawluk, S. Mineralogical composition of some Grey Wooded soils developed from glacial till. Can. J. Soil Sci. 41:228-240. 1961.

65. Raudnitz, H. Occurrence of humic acids in leaves. Chem. Ind. 1957. No. 51:1650-1651.

66. Rennie, D. A., E. Truog and O. N. Allen. Soil aggregation as influenced by microbial gums, level of fertility and kind of crop. Soil Sci. Soc. Amer. Proc. 18:399-403. 1954.

67. Russell, E. W. Soil conditions and plant growth. 9th ed. Longmans, Green and Co., 137 Bond Street, Toronto 2. 1961.

68. Savage, S. M. and F. J. Stevenson. Behavior of soil humic acids toward oxidation with hydrogen peroxide. Soil Sci. Soc. Amer. Proc. 25:35-39. 1961.

69. Scheffer, E. and R. Kickuth. Chemische Abbauversuche an einer näturlichen Huminsäure. Mitteilung I. Z. Pflanzenernähr., Dung., Bodenk. 94(139):180-188. 1961.

70. Scheffer, E. and R. Kickuth. Chemische Abbauversuche an einer näturlichen Huminsäure. Mitteilung II. Z. Pflanzenernähr., Dung., Bodenk. 94(139):188-198. 1961.

71. Schnitzer, M. and W. A. Delong. Investigations on the mobilization and transport of iron in forested soils. II. The nature of the reaction of leaf extracts and leachates with iron. Soil Sci. Amer. Proc. 19:363-368. 1955.

72. Schnitzer, M., D. A. Shearer and J. R. Wright. A study in the infrared of high-molecular weight organic matter extracted by various reagents from a Podzolic B. Horizon. Soil Sci. 87:252-257. 1959.

73. Schnitzer, M., J. R. Wright and J. G. Desjardins. A comparison of the effectiveness of various extractants for organic matter from two horizons of a podzol profile. Can. J. Soil Sci. 38:49-53. 1958.

74. Schnitzer, M. and J. R. Wright. Nitric acid oxidation of the organic matter of a podzol. Soil Sci. Soc. Amer. Proc. 24:273-276. 1960.

75. Shapiro, L. and W. W. Brannock. Rapid analysis of silicate rocks. Geol. Survey Bull. 1036-C Revised Circ. U. S. Gov't. Printing Office, Washington, D.C. 1956.

76. Sowden, F. J. Note on the occurrence of amino groups in soil organic matter. Can. J. Soil Sci. 37:143-144. 1957.

77. Sowden, F. J., D. I. Parker and J. G. Atkinson. Comparison of organic matter fractions from three soil types. Sci. Agr. 32:127-142. 1952.

78. St. Arnaud, R. J. The micro-Kjeldahl method for the determination of nitrogen in soils. Manual of Laboratory Methods, Dep. Soil Sci. Univ. Saskatchewan. 1960.

79. Steelink, C., J. W. Berry, A. Ho and H. E. Nordby. Alkaline degradation products of soil humic acid. Sci. Proc. Royal Dublin Soc. Series A. 1:59-67. 1960.

80. Stevenson, F. J., J. D. Mark, J. E. Varner and W. P. Martin. Electrophoretic and chromatographic investigations of clay absorbed organic colloids. I. Preliminary investigations. Soil Sci. Soc. Amer. Proc. 16:69-73. 1950.

81. Swaby, R. J. Soil organic matter. C.S.I.R.O. 10th Annual Report. Government Printers, Sydney. 1958.

82. Technical Association of the Pulp and Paper Industry. Methoxyl groups in wood. TAPPI T2 m-43. 122 East 42nd St., New York 17, N. Y. 1949.

83. Thorp, J., L. E. Strong and E. Gamble. Experiments in soil genesis. The role of leaching. Soil Sci. Soc. Amer. Proc. 21:99-102. 1957.

84. Toogood, J. A. and D. L. Lynch. Effect of cropping systems and fertilizers on mean weight-diameter of aggregates of Breton plot soil. Can. J. Soil Sci. 83:151-156. 1959.

85. Tyurin, I. V. Some results of work on the comparative study of the composition of humus in soils of the U. S. S. R. Soils Fert. 16:436. 1953.

86. U. S. D. A. Soil Survey Manual. Handbook No. 18. U. S. Government Printing Office, Washington, D.C.

87. VanSlyke, D. D. and J. Folch. Manometric carbon determination. J. Biol. Chem. 136:509-541. 1940.

88. VanSlyke, D. D. and J. M. Neill. The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. I. J. Biol. Chem. 61:523-574. 1924.

89. VanSlyke, D. D., J. Plazin and J. R. Weisiger. Reagents for the VanSlyke-Folch wet carbon combustion. J. Biol. Chem. 191:299-304. 1951.

90. Waksman, S. A. Humus. The Williams and Wilkins Company, Baltimore. 1936.

91. Waksman, S. A. and K. N. R. Iyer. Contribution to our knowledge of the chemical nature and origin of humus. I. On the synthesis of the "humus nucleus". Soil Sci. 34:43-67. 1932.

92. Waksman, S. A. and K. R. Stevens. Critical study of the methods for determining the nature and abundance of soil organic matter. Soil Sci. 63:251-264. 1947.

93. Wright, J. R. and M. Schnitzer. Alkaline permanganate oxidation of the organic matter of the Ao and B_{21} horizons of a podzol. Can. J. Soil Sci. 39:44-53. 1959.

94. Wright, J. R. and M. Schnitzer. Oxygen-containing functional groups in the organic matter of a podzol soil. Nature 184:1462-1463. 1959.

95. Wright, J. R. and M. Schnitzer. An estimate of the aromaticity of the organic matter of a podzol soil. Nature 190:703-704. 1961.

96. Wright, J. R., M. Schnitzer and R. Levick. Some characteristics of the organic matter extracted by dilute inorganic acids from a podzolic B horizon. Can. J. Soil Sci. 38:14-22. 1958.

97. Wood, J. C., S. E. Moschopedis and R. M. Elofson. Studies in humic acid chemistry. I. Molecular weights of humic acids in sulpholane. Fuel XL:193-201. 1961.

98. Zeichmann, W. and B. Meyer. Zur chemic der Huminsäure-Vorstufen. Trans. 7th Intern. Congr. Soil Sci. Vol. II:105-111. 1960.